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(57) Abstract

The anticancer drug taxol binds to beta-tubulin in assembled microtubules (MT) and causes cell cycle arrest in animal cells; in contrast, the effect of taxol varies in fungi. For instance, the taxol-producer *Pestalotiopsis microspora* Ne32, an ascomycete, is resistant to taxol ($1C_{50}$>11.7 M), whereas *Pythium ultimum* and *Phytophthora cinnamomi*, two oomycetes, are sensitive to taxol (IC_{50} 0.1 μM). cDNAs encoding beta-tubulin from *P.microspora*, *P. ultimum*, and *P. cinnamomi* were isolated. The deduced amino acid sequences of beta-tubulin from *P. microspora*, *P.ultimum*, and *P. cinnamomi* can be used in (1) binding assays for the detection of taxol and taxol-like substances; (2) diagnostic assays for the pharmacologic efficacy of taxol against a tumor sample; (3) designing drugs with taxol-like activity via application of information regarding the effect of specific residues on taxol binding; and (4) detection of taxol and taxol-like activity via use of taxol-sensitive and taxol-resistant isogenic strains of *P.ultimum* constructed by substitution of residues necessary for taxol binding.

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FUNGAL BETA-TUBULIN GENES

TECHNICAL FIELD OF THE INVENTION

The invention relates to a bio-affecting composition and to a biological diagnostic and experimental agent.

BACKGROUND OF THE INVENTION

TAXOL® (Bristol-Myers-Squibb), generically known as paclitaxel (hereinafter referred to as "taxol"), is a complex diterpenoid which has demonstrated anti-tumor activity against breast and ovarian cancer (Rowinsky, E.K. and Donehower, R.C. 1991. "The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics," *Pharmacol Ther* 52:35-84). Its anti-tumor activity is due to its ability to bind to beta-tubulin in assembled microtubules (MTs) and stabilize them (Manfredi, J.J. and Horwitz, S.B. 1984. "Taxol: an antimitotic agent with a new mechanism of action," *Pharmacol Ther* 25:83-125; and Horwitz, S.B. 1992. "Mechanism of action of taxol," *Trends Pharmacol Sci* 13:134-136). *In vivo,* taxol affects spindle function during mitosis, resulting in cell cycle arrest in G2/M phase. *In vitro,* taxol promotes MT assembly and prevents their disassembly under conditions which would otherwise cause depolymerization (Schiff, et al. 1979. "Promotion of microtubule assembly in vitro by taxol" *Nature* 277:665-667; and Pamess, J. and Horwitz, S.B. 1981 "Taxol binds to polymerized tubulin *in vitro*," *J Cell Biol* 91:479-487). The taxol binding site on beta-tubulin has been characterized by photo cross-labeling, electron crystallography, and mutagenesis, and involves several regions of beta-tubulin (Rao, et al. 1994. "3'-(p-Azidobenzamido) taxol photolabels the N-terminal 31 amino acids of β-tubulin," *J Biol Chem* 269:3132-3134; Rao, et al. 1995. "Characterization of the taxol binding site on the microtubule," *J Biol Chem* 270:20235-20238; Nogales, et al. 1998. "Structure of the αβ tubulin dimer by electron crystallography," *Nature* 391:199-203; Nogales, et al. 1999. "High-resolution model of the microtubule," *Cell* 95:79-88; and Giannakakou, et al. 1997. "Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization," *J Biol Chem* 272:17118-17125).

Taxol was found originally in the inner bark of pacific yew trees *(Taxus brevifolia)* by Wani et al. (Wani, et al. 1971. "Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia," J Am Chem Soc* 93:2325-2327), and noted to constitute about 0.02% of dry phloem weight. The limited resource of yew trees made it advantageous to locate additional sources for taxol.

In 1993, Stierle et al. reported the isolation of a taxol-producing fungus, *Taxomyces andreanae,* an endophyte associated with *T. brevifolia* (Stierle, et al. 1993. "Taxol and taxane production by *Taxomyces andreanae,* an endophytic fungus of pacific yew," *Science* 260:214-216). *T. andreanae* produces authentic taxol, though at very low levels (24-50 ng/liter of liquid culture). Recently, additional taxol-producing fungi have been isolated, including various strains of *Pestalotiopsis microspora* (Li, et al. 1996. "Endophytic taxol-producing fungi from bald cypress, *Taxodium distichum," Microbiolog* 142:223-226; Li, et al. 1998. "The induction of taxol production in the endophytic *fungus-Periconia* sp. from *Torreya grandifolia," J Ind Microbiol Biotechnol* 20:259-264; Strobel, et al. 1996. "Taxol from fungal endophytes and the issue of biodiversity," *J Ind Microbiol* 17:417-423; and Strobel, et al. 1996. "Taxol from *Pestalotiopsis microspora,* an endophytic fungus of *Taxus wallachiana," Microbiolog* 142:435-440). One *P. microspora* strain, Ne32, was isolated from the inner bark of Himalayan yew *T. wallachiana,* and produces approximately 50 μg taxol per liter of liquid culture (Strobel, et al. 1996. *Microbiolog* 142:435-440; and Li, et al. 1998. "Stimulation of taxol production in liquid culture of *Pestalotiopsis microspora," Mycol Res* 102:461-464). The taxol produced from fungal sources has been reported to be spectroscopically and chromatographically identical to taxol isolated from yew trees, and has shown similar pharmacological effects on cancer cell lines (Strobel, et al. 1996. *Microbiolog* 142:435-440). The production of taxol from fungal sources has provided broader resources of taxol, reduced production costs, and a means of meeting the increasing demand for taxol.

While taxol has been shown to be toxic to cells from a wide range of organisms including mammals, sea urchin, *Drosophila, Xenopus, Physarum, Haemanthus,* and *Trypanosoma* (Baum, et al. 1981. "Taxol, a microtubule stabilizing agent, blocks the replication of *Trypanosoma cruzi," Proc Natl Acad Sci USA* 78:4571-4575; Kellogg, et al. 1989. "Proteins in the centrosome, spindle, and kinetochore of the early *Drosophila*

embryo," *J Cell Biol* 109:2977-2991; and Manfredi, J.J. and Horwitz, S.B. 1984. *Pharmacol Ther* 25:83-125), variable sensitivity to taxol has been reported in fungi. Young et al. tested taxol toxicity on representative species from different fungal groups (Young, et al. 1992. "Antifungal properties of taxol and various analogues," *Experientia* 48:882-885). In Young's study, five oomycete species were identified as sensitive to taxol (IC_{50} 0.4-5.9 μM), including the plant pathogens *Pythium ultimum* and *Phytophthora capsici*. In *P. capsici*, taxol inhibited nuclear division at low concentrations, indicating that it acts through a mechanism similar to that in mammalian cells. In contrast, four ascomycete species were identified as resistant to taxol (IC_{50} > 50 μM). This resistance was reported to be due to the reduced ability of fungal microtubules to interact with taxol. Taxol was also shown to be unable to stabilize MTs assembled with purified *S. cerevisiae* tubulin (Bames, et al. 1992. "Yeast proteins associated with microtubules *in vitro* and *in vivo*," *Mol Biol Cell* 3:29-47) and only weakly stabilize MTs from *Aspergillus nidulans* (Yoon, Y. and Oakley, B.R. 1995. "Purification and characterization of assembly-competent tubulin from *Aspergillus nidulans*," *Biochem* 34:6373-6381).

Because of the anticancer properties of taxol and the variability of fungi to taxol sensitivity, there is a continuing need for isolating and/or identifying novel beta-tubulin genes useful for developing isogenic fungal strains that are either taxol-sensitive or taxol-resistant. These beta-tubulin genes and/or isogenic fungal strains can then be applied to anticancer drug screening and for developing diagnostic tests for tumor sensitivity assays.

SUMMARY OF THE INVENTION

In one aspect, the invention is a purified DNA segment encoding a beta-tubulin of the fungal species *Pestalotiopsis microspora* or a portion thereof. Preferably, the DNA segment encodes at least one taxol binding site. For some uses, it is preferable that the DNA segment encodes a protein having taxol binding site I and taxol binding site II. For DNA segments that encode proteins which function as beta-tubulins, the DNA segment encodes a protein which has taxol binding site I and taxol binding site II and is able to interact with alpha-tubulin. An exemplary DNA segment comprises at least a portion of SEQ ID NO:1. Another exemplary DNA segment comprises a portion of SEQ ID NO:1 comprising the nucleotide sequence from nucleotide 75 through nucleotide 167 of SEQ ID NO:1, with or without substitution Another exemplary DNA segment comprises a portion of SEQ ID

NO:1 comprising the nucleotide sequence from nucleotide 708 through nucleotide 764 of SEQ ID NO:1, with or without substitution. Another exemplary DNA segment comprises a portion of SEQ ID NO:1 comprising the nucleotide sequence from nucleotide 708 through nucleotide 764 of SEQ ID NO:1, wherein either nucleotide 729, nucleotide 730 or nucleotide 731 or mixtures thereof are substituted. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1 wherein the DNA segment encodes a beta-tubulin. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1 wherein at least one nucleotide in the nucleotide sequence is substituted and wherein the taxol binding capacity of the beta-tubulin is not altered. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1 wherein at least one nucleotide in the nucleotide sequence is substituted and wherein the taxol binding capacity of the beta-tubulin is altered.

In another aspect, the invention is an amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pestalotiopsis microspora*. Preferably, the amino acid sequence comprises at least one taxol binding site. For some uses, it is preferable that the amino acid sequence is a protein having taxol binding site I and taxol binding site II. For amino acid sequences that can function as beta-tubulins, the amino acid sequence has taxol binding site I and taxol binding site II and is able to interact with alpha-tubulin. An exemplary amino acid sequence comprises at least a portion of the beta-tubulin as depicted in SEQ ID NO:2. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 comprising Amino Acids 1-31, with or without substitution. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 comprising Amino Acids 212-230, with or without substitution. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 comprising Amino Acids 212-230 with an amino acid substitution at Amino Acid 219. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 consisting essentially of Amino Acids 1-446 wherein the portion behaves as a taxol-resistant beta-tubulin. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 consisting essentially of Amino Acids 1-446 the portion contains at least one amino acid substitution that alters the taxol binding property of the portion. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:2 consisting essentially of Amino Acids 1-446 the portion contains at least one amino acid

substitution that does not alters the taxol binding property of the portion. Another exemplary amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of the amino acid sequence surrounding Amino Acid 219 as numbered in SEQ ID NO:2.

In another aspect, the invention is a purified DNA segment encoding a beta-tubulin of the fungal species *Pythium ultimum* or a portion thereof. Preferably, the DNA segment encodes at least one taxol binding site. For some uses, it is preferable that the DNA segment encodes a protein having taxol binding site I and taxol binding site II. For DNA segments that encode proteins which function as beta-tubulins, the DNA segment encodes a protein which has taxol binding site I and taxol binding site II and is able to interact with alpha-tubulin. An exemplary DNA segment comprises at least a portion of SEQ ID NO:3. Another exemplary DNA segment comprises a portion of SEQ ID NO:3 comprising nucleotide 92 through nucleotide 184, with or without substitution. Another exemplary DNA segment comprises a portion of SEQ ID NO:3 comprising the nucleotide sequence from nucleotide 725 through nucleotide 781, with or without substitution. Another exemplary DNA segment comprises a portion of SEQ ID NO:3 comprising the nucleotide sequence from nucleotide 725 through nucleotide 781, wherein either nucleotide 746, nucleotide 747 or nucleotide 748 or mixtures thereof are substituted. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3, wherein the DNA segment encodes a beta-tubulin. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3 with at least one nucleotide substitution in the nucleotide sequence and wherein the taxol binding capacity of the beta-tubulin is not altered. Another exemplary DNA segment comprises the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3 with at least one nucleotide substitution in the nucleotide sequence and wherein the taxol binding capacity of the beta-tubulin is altered.

In another aspect, the invention is an amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pythium ultimum*. Preferably, the amino acid sequence comprises at least one taxol binding site. For some uses, it is preferable that the amino acid sequence is a protein having taxol binding site I and taxol binding site II. For amino acid sequences that can function as beta-tubulins, the amino acid sequence has taxol

binding site I and taxol binding site II and is able to interact with alpha-tubulin. An exemplary amino acid sequence comprises at least a portion of the beta-tubulin as depicted in SEQ ID NO:4. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 comprising Amino Acids 1-31, with or without substitution. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 comprising Amino Acids 212-230, with or without substitution Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 comprising Amino Acids 212-230, wherein the amino acid at Amino Acid 219 is substituted. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 consisting essentially of Amino Acids 1-446 and wherein the portion behaves as a taxol-sensitive beta-tubulin. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 consisting essentially of Amino Acids 1-446 having at least one amino acid substitution that alters the taxol binding property of the portion. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:4 consisting essentially of Amino Acids 1-446 having at least one amino acid substitution that does not alter the taxol binding property of the portion. Another exemplary amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of the amino acid sequence surrounding Amino Acid 219 as numbered in SEQ ID NO:4.

In another aspect, the invention is a purified DNA segment encoding a beta-tubulin of the fungal species *Phytophthora cinnamomi* or a portion thereof, wherein the DNA segment consists essentially of at least a portion of SEQ ID NO:5. An exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 through nucleotide 103. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 through nucleotide 103, wherein at least one nucleotide in the nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code nucleotide 80 cannot consist of adenine while nucleotide 81 is thymine and nucleotide 82 is adenine, cytosine or thymine. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising nucleotide sequence from nucleotide 644 through nucleotide 700. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising nucleotide sequence from nucleotide 644 through nucleotide 700, wherein at least one nucleotide in the nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code nucleotide 665 cannot be adenine while

nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 to nucleotide 1342 and wherein the DNA segment encodes a beta-tubulin. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 to nucleotide 1342, wherein at least one nucleotide in the nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code, nucleotide 665 cannot be adenine while nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 to nucleotide 1342, wherein at least one nucleotide in the nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code, nucleotide 665 cannot be adenine while nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine, and wherein the taxol binding capacity of the beta-tubulin is not altered. Another exemplary DNA segment comprises a portion of SEQ ID NO:5 comprising the nucleotide sequence from nucleotide 11 to nucleotide 1342, wherein at least one nucleotide in the nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code, nucleotide 665 cannot be adenine while nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine, and wherein the taxol binding capacity of the beta-tubulin is altered.

In another aspect, the invention is an amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Phytophthora cinnamomi* as depicted in SEQ ID NO:6. An exemplary amino acid sequence comprises a portion of SEQ ID NO:6 comprising Amino Acids 1-31. An exemplary amino acid sequence comprises a portion of SEQ ID NO:6 comprising Amino Acids 1-31, having at least one amino acid substituted, providing that when only one amino acid is substituted Amino Acid 24 is not isoleucine. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:6 comprising Amino Acids 212-230. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:6 comprising Amino Acids 212-230, having at least one amino acid substituted, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:6 comprising Amino Acids 212-230 with an amino acid substitution at Amino Acid 219, wherein the Amino Acid 219 is not substituted with asparagine. Another exemplary amino acid

sequence comprises a portion of SEQ ID NO:6 consisting essentially of Amino Acids 1-446, wherein the portion behaves as a taxol-sensitive beta-tubulin. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:6 consisting essentially of Amino Acids 1-446, wherein the portion contains at least one amino acid substitution, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine, and wherein the amino acid substitution alters the taxol binding property of the portion. Another exemplary amino acid sequence comprises a portion of SEQ ID NO:6 consisting essentially of Amino Acids 1-446, wherein the portion contains at least one amino acid substitution, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine, and wherein the amino acid substitution does not alter the taxol binding property of the portion. Another exemplary amino acid sequence is substituted at Amino Acid 219 with any amino acid except asparagine which perturbs the three-dimensional structure of the amino acid sequence surrounding Amino Acid 219.

In another aspect, the invention is a vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Pestalotiopsis microspora* or a portion thereof. Preferably, the vector comprises a portion encoding at least one taxol binding site.

In another aspect, the invention is a vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Pythium ultimum* or a portion thereof. Preferably, the vector comprises a portion encoding at least one taxol binding site.

In another aspect, the invention is a vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Phytophthora cinnamomi* wherein the DNA segment consists essentially of SEQ ID NO:5 or a portion thereof. Preferably, the vector comprises a portion encoding at least one taxol binding site.

In another aspect, the invention is a method of determining the taxol binding capacity of a beta-tubulin or beta-tubulin-like compound comprising providing antibodies raised against amino acid sequences comprising at least one taxol binding site of a beta-tubulin from a taxol-resistant *Pestalotiopsis microspora,* a taxol-sensitive *Pythium ultimum,* or taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6 to form a reagent, such antibodies distinguishing between taxol-binding and non-taxol-binding properties; contacting the beta-tubulin or beta-tubulin-like compound with the reagent; and determining

degree of binding between the antibodies in the reagent and the beta-tubulin or beta-tubulin-like compound; whereby binding of antibodies raised against a taxol-resistant *Pestalotiopsis microspora* to the beta-tubulin or beta-tubulin-like compound indicates taxol resistance and whereby binding of antibodies which specifically recognize taxol-binding properties indicate taxol sensitive; whereby binding of antibodies which specifically recognize taxol-non-binding properties indicate taxol resistance. In one embodiment, the antibodies in the reagent are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-resistant *Pestalotiopsis microspora.* In another embodiment, the antibodies in the reagent are raised against an amino acid sequence comprises at least one taxol binding site from SEQ ID NO:2. In another embodiment, the antibodies in the reagent are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-sensitive *Phythium ultimum.* In another embodiment, the antibodies in the reagent are raised against an amino acid sequence comprises at least one taxol binding site from SEQ ID NO:4. In another embodiment, the antibodies in the reagent are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6. In another embodiment, the antibodies in the reagent are raised against an amino acid sequence comprises at least one taxol binding site from SEQ ID NO:678. In this method, the beta-tubulin or beta-tubulin-like compound are selected from the group consisting of recombinantly expressed protein, exogenously isolated protein, synthetic peptides, and cell cultures.

In another aspect, the invention is a method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising providing beta-tubulins with amino acid sequences comprising both taxol binding sites from *Pythium ultimum* or taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6 in addition to alpha-tubulin from any taxol-sensitive organism to form a reagent; contacting the composition of matter with the reagent; and determining the ability of the composition of matter to promote MT assembly or ability to prevent depolymerization of assembled MTs under depolymerizing conditions;whereby the ability to promote microtubule assembly or prevent depolymerization indicate the possible presence of taxol or taxol-like compounds in the composition of matter.

In another aspect, the invention is a method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising providing mycelia of taxol-sensitive *Pythium ultimum* or a taxol-sensitive *Phytophthora cinnamomi* which harbors beta-tubulin in SEQ ID NO:6; contacting the composition of matter with the mycelia in the presence of the labeled taxol; and determining the degree of competitive inhibition of binding between the beta-tubulins and the labeled taxol by the composition of matter, whereby the composition of matter is determined to possess taxol or taxol-like compounds if it is able to block labeled taxol binding to the beta-tubulins from the taxol-sensitive *Pythium ultimum* or *Phytophthora cinnamomi.*

In another aspect, the invention is a method of altering the taxol binding property of a recombinantly expressed beta-tubulin or a portion thereof comprising determining the identity of the codon at Amino Acid 219 as numbered in SEQ ID NO:1 in the coding region of the vector; and if the codon at Amino Acid 219 codes for any amino acid except threonine, substituting nucleotides in the codon to code for threonine at Amino Acid 219 to alter a non-taxol-binding beta-tubulin or portion thereof to a taxol-binding beta-tubulin or portion thereof, or if the codon at Amino Acid 219 codes for threonine, substituting nucleotides in the codon to code for any amino acid except threonine at Amino Acid 219 to alter a taxol-binding beta-tubulin or portion thereof to a non-taxol-binding beta-tubulin or portion thereof.

In another aspect, the invention is a method of developing a taxol-sensitive fungal cell from a taxol-resistant fungal cell comprising transforming the non-taxol-sensitive fungal cell by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2; wherein the transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

In another aspect, the invention is a transgenic taxol-sensitive fungal cell transformed by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2, wherein the transformed fungal cell expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

In another aspect, the invention is a method of developing a taxol-resistant fungal cell from a taxol-sensitive fungal cell comprising transforming the taxol-sensitive fungal cell by introducing a DNA segment encoding non-taxol-binding beta-tubulin wherein the amino acid at Amino Acid 219 as numbered in SEQ ID NO:2 is not threonine; wherein the transformed fungal cell over-expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

In another aspect, the invention is a transgenic taxol-sensitive fungal cell transformed by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2, wherein the transformed fungal cell over-expresses the DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

In another aspect, the invention is a method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising providing distinguishable taxol-resistant and taxol-sensitive fungal cells; contacting the composition of matter with the fungal cells; and determining the growth of inhibition of the fungal cells; whereby the composition of matter is determined to possess taxol or taxol-like compounds if it is able to inhibit the growth of taxol-sensitive fungal cells but not able to inhibit the growth of taxol-resistant fungal cells. The method can be performed wherein the distinguishable taxol-resistant and taxol-sensitive fungal cells consists essentially of transgenic taxol-resistant and taxol-sensitive isogenic fungal cells. The method can also be performed with taxol-resistant fungal cells derived from one fungus which is unrelated to the fungi from which the taxol-sensitive fungal cells are derived.

In another aspect, the invention is a method for controlling the growth of a plant pathogen comprising determining the taxol sensitivity of the plant pathogen; and if the pathogen is determined to be taxol-sensitive, the plant and soil surrounding the plant are treated with a taxol-producing *P. microspora*. In an exemplary method, the taxol sensitivity of the plant pathogen is determined by identifying Amino Acid 219, wherein the plant is designated as taxol-sensitive if Amino Acid 219 is threonine.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph depicting the effect of taxol on mycelial growth in *P. microspora, P. ultimum, P. cinnamomi* and *A. klebsiana.* Fungal mycelia were grown on potato dextrose agar (PDA) plates containing different concentrations of taxol. The inhibitory effect of taxol was assessed by colony diameter, and compared to mycelia grown in the absence of taxol. Experiments were conducted in duplicate, and data presented are an average of several experiments.

Fig. 2 depicts the nucleotide and deduced amino acid sequence of beta-tubulin from *P. microspora* Ne32 cDNA, TUBB-pm. Numerals on the left indicate nucleotide position, and numerals on the right indicate amino acid position. The sequences of the gene-specific primers NETUB5 and NETUB6 are underlined. The translation initiation codon ATG is underlined, the translation termination codon is marked by an asterisk, and the putative polyadenylation signal is double underlined.

Fig. 3 depicts the nucleotide and deduced amino acid sequence of beta-tubulin from *P. ultimum* cDNA, TUBB-pu. Numerals on the left indicate nucleotide position, and numerals on the right indicate amino acid position. The sequences of the gene-specific primers WT1L-U and WT1L-L are underlined. The translation initiation codon ATG is underlined, the translation termination codon is marked by an asterisk, and the two putative polyadenylation signals are double underlined. The arrow at nucleotide 1507 indicates the position of the poly (A) tract in the shorter 1537 bp cDNA.

Fig. 4 depicts the nucleotide and deduced amino acid sequence of beta-tubulin from *P. cinnamomi* cDNA, TUBB-pc. Numerals on the left indicate nucleotide position, and numerals on the right indicate amino acid position. The sequences of the gene-specific primers PCBTUB1U, PCBTUB2U and PCBTUB4L are underlined. The translation initiation codon ATG is marked by ###, and the translation termination codon is marked by an asterisk. Nucleotides and amino acids which differ between TUBB-pc and the sequence U22050 (Genbank accession number) reported by Weerakoon et al. (Weerakoon et al. 1998. "Isolation and characterization of the single β-tubulin gene in *Phytophthora cinnamomi,*" *Mycologia* 90:85-95) are double underlined.

Fig. 5 compares the amino acid sequence of *P. cinnamomi* beta-tubulins ("TUBB-pc") reported herein and previously by Weerakoon et al. (Weerakoon, et al. 1998. *Mycologia* 90:85-95) ("U22050"). The deduced amino acid sequence of *P. cinnamomi* TUBB-pc is shown in its entirety, and the eight residues (Amino Acids 24, 219, 249, 251-253, 359, and 428) which differ from the U22050 sequence (Genbank accession number) reported by Weerakoon et al. (1998) are shown below the TUBB-pc sequence. Amino Acids 1-31 and 212-231 (denoted herein as taxol binding region I and II, respectively) are indicated by a line above the sequence.

Fig. 6A and 6B depict the amino acid sequence alignment of beta-tubulins. The alignment was obtained using the ClustalW alignment program. The amino acid sequence of *P. microspora* beta-tubulin is shown in its entirety, and residues which differ in other beta-tubulins are shown below. Numerals on the right indicate amino acid positions. Sequences underlined indicate regions important for GTP binding (Amino Acids 63-77), phosphate binding (Amino Acids 140-146), and Mg^{2+} binding (Amino Acids 203-206). Amino Acids 1-31 and 212-231 (denoted here as taxol binding region I and II, respectively) are indicated by a line above the sequence. Amino Acids Phe270, Leu273 and Ser364 are marked above with #. Amino acids which are important for fungal resistance to benzimidazoles (Amino Acids 6, 165, 167, 198, 200 and 241) are marked above by asterisks. Gaps in alignment are indicated by dashes, and the end of each sequence is marked by "$". The Genbank accession numbers for beta-tubulins from *N. crassa, A. nidulans* benA, *A. klebsiana* and human β2 are listed in Table I. The *P. cinnamomi* depicted is SEQ ID NO:6.

Fig. 7A and 7B are graphs depicting the specific binding of [^{3}H]taxol to *P. ultimum* but not to *P. microspora*. Fig. 7A demonstrates that specific binding of [^{3}H]taxol to *P. ultimum* increased as a function of [^{3}H]taxol concentration, while *P. microspora* showed no or very little specific binding. Actively growing fungal cells were incubated with different concentrations of [^{3}H]taxol at room temperature for 2 hours before quenching. Specific binding was calculated as the difference between binding of [^{3}H]taxol in the presence and absence of a 100-fold excess of unlabeled taxol. Specific binding represents 30-70% of the total binding to *P. ultimum* but less than 5% of the total binding to *P. microspora*. Binding of [^{3}H]taxol to *P. microspora* cells was performed in the presence of Triton X-100 (0.1%

v/v) to disrupt the cell membrane. Fig. 7B depicts the reduction of specific binding of [^{3}H]taxol to *P. ultimum* in the presence and absence of the microtubule-depolymerizing drug thiabendazole in a dose-dependent manner. Cells were either not treated (0 μM) or treated with different concentrations of thiabendazole for three hours at room temperature. Subsequently, cells were incubated with 5 nM [^{3}H]taxol in the presence or absence of a 100-fold excess of unlabeled taxol for two hours before quenching. The specific binding of [^{3}H]taxol to untreated cells was defined as 100%. The experiments depicted in Fig. 7A and 7B were conducted in duplicate, and data presented are representative of several experiments.

Fig. 8 depicts the amino acid sequences of the taxol binding region I (Amino Acids 1-31) and II (Amino Acids 212-231) of beta-tubulins from different organisms. The amino acid sequences of the taxol binding regions I and II for pig beta-tubulin are shown in their entirety and residues which differ are shown for other beta-tubulins. The taxol sensitivity of each organism is indicated, "s" for sensitive and "r" for resistant. Amino Acids 15-25 and 212-222, which have been shown to be involved in taxol binding by both cross-linking and electron crystallography, are marked with asterisks. The taxol binding region II of *A. klebsiana* is between Amino Acids 211-230 due to a gap in its sequence. Pig beta-tubulin is described by Nogales, et al. (Nogales, et al. 1999. *Nature* 391:199-203), and Genbank accession numbers for other sequences are listed in Table I. The sequence for *P. cinnamomi* presented herein is depicted in SEQ ID NO:6.

DETAILED DESCRIPTION

One aspect of the present invention is an isolated gene comprising an open reading frame coding for the protein beta-tubulin or a portion thereof. The corresponding cDNA have been isolated and characterized for taxol-resistant *Pestalotiopsis microspora* Ne32, taxol-sensitive *Pythium ultimum*, and taxol-sensitive *Pythium cinnamomi*. The nucleotide and deduced amino acid sequences of beta-tubulin for *Pestalotiopsis microspora* Ne32 are given in SEQ ID NO:1 and SEQ ID NO:2, respectively; for *Pythium ultimum*, in SEQ ID NO:3 and SEQ ID NO:4, respectively; and for *Pythium cinnamomi*, in SEQ ID NO:5 and SEQ ID NO:6, respectively. Through characterization of the taxol sensitivity of the beta-tubulins encoded by the genes of the present invention, it has been found that the identity of Amino Acid 219 of beta-tubulin as numbered in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID

NO:6 is an indicator of taxol sensitivity. The presence of threonine at Amino Acid 219 ("Thr219") indicates taxol sensitivity, while the presence of asparagine ("Asn219") or glutamine ("Gln219") indicate taxol resistance.

In another aspect, the present invention is the beta-tubulin protein or protein fragments encoded by the novel genes disclosed herein. Since the *P. ultimum* and *P. cinnamomi* beta-tubulin proteins of the present invention are capable of binding taxol, proteins and protein fragments comprising taxol-binding sites derived from the genes coding for beta-tubulin described herein can be produced by heterologous expression in *E. coli* and other systems, purified by standard procedures, and used in an *in vitro* assay for detecting taxol and taxol-like substances by using methods well known in the art (Schiff, et al. 1979. *Nature* 277:665-667). For example, the beta-tubulin proteins of the present invention can be used to screen plant or fungal extracts as well as synthetic compounds for taxol or taxol-like substances as possible anticancer drugs. Beta-tubulins produced by making specific amino acid substitutions, deletions, or alterations can be used as experimental tools to further determine the molecular basis of taxol binding to the beta-tubulin protein.

In another aspect, antibodies (polyclonal or monoclonal) raised against all or portions of the beta tubulins of the present invention can be used to determine if a composition of matter has taxol binding properties. In one method, antibodies capable of binding to taxol-sensitive beta-tubulin and/or taxol-resistant beta tubulins are exposed to a composition of matter prepared for *in situ* hybridization (Ausubel, et al. 1997. *Current Protocols in Molecular Biology*, John Wiley & Sons), Elisa, or Western blot. Visualization of antibody-antigen binding is mediated through any means known in the art, e.g., secondary radiolabeled or fluorescent antibodies or colorimetric methods using peroxidase and/or alkaline phosphatase (Harlow, E.D. and Lane, D. 1988. *Antibodies: A Laboratory Manual*). For example, antibodies raised to a portion of SEQ ID NO:4 comprising Amino Acid 219 would bind to a beta-tubulin which had threonine at Amino Acid 219 but would not bind to a beta-tubulin having a different amino acid at Amino Acid 219, so that detectable binding would indicate the presence of threonine at Amino Acid 219, and hence, sensitivity to taxol. This type of assay is useful for screening a variety of compositions of matter, including living matter such as plants or microorganisms, or non-living matter such as plant materials, patient samples, or compound libraries for the presence of beta-tubulin.

In another aspect, the present invention is a method of designing taxol analogs or other compounds which mimic the interaction of taxol with beta-tubulin based on the identification of specific amino acids in the beta-tubulins corresponding to taxol-binding and taxol-sensitivity. The previously reported three-dimensional structure (Nogales, et al. 1998. *Nature* 391:199-203) can be applied to developing and optimizing antineoplastic and antifungal compounds with respect to Amino Acid 219 and the surrounding area. Further, such information can also be used to generate mutant beta-tubulins with altered taxol sensitivity by substituting amino acids at specific positions in the beta-tubulin protein.

In another aspect, the present invention is a method of generating isogenic strains of fungi using a gene of the present invention, which allows studies of taxol related pharmacology to be performed against a known background. Further, the present invention is a method of using these isogenic fungal strains, one of which is taxol sensitive and the other taxol resistant, to screen plant extracts, fungal extracts, extracts from other organisms, and synthetic compounds for taxol-like substances as possible anticancer agents. The present invention is also a method of using two unrelated fungal strains, one of which is taxol sensitive and the other taxol resistant, to screen plant extracts, fungal extracts, extracts from other organisms, and synthetic compounds for taxol-like substances as possible anticancer agents.

The genes and proteins of the present invention are characterized in the following examples. It is to be understood that the examples are exemplary of the invention and are intended to be illustrative of the invention, but are not to be construed to limit the scope of the invention in any way. Modifications may be made in the structural features of the invention without departing from the scope of the invention.

Example 1: Differential taxol sensitivity in selected fungi

Taxol sensitivity was established for the fungal strains used in the isolation of the beta-tubulin cDNAs of the present invention.

Pestalotiopsis microspora strain Ne32, previously disclosed in U.S. Patent No. 5,861,302, was licensed from Montana State University. *Pythium ultimum* (ATCC 26083), *Achlya klebsiana* (ATCC 52605), and *Pythium cinnamomi* (ATCC 200982) were purchased

from American Type Culture Collection (Manassas, VA). Taxol was obtained from Sigma Chemical Company (St. Louis, MO).

The effect of taxol on the growth of *P. microspora* Ne32, *P. ultimum,* and *P. cinnamomi* was examined. For comparison, *A. klebsiana,* an oomycete closely related to *P. ultimum,* was also included in these experiments. As shown in Fig. 1, the growth of *P. microspora* was highly resistant to taxol up to 11.7 μM. By comparison, *A. klebsiana* showed moderate sensitivity, and its growth was reduced by 40% in 11.7 μM taxol. Finally, *P. ultimum* and *P. cinnamomi* were shown to be the most sensitive of the four species. Growth of *P. ultimum* and *P. cinnamomi* was inhibited even at low concentrations of taxol (IC_{50} 0.1 μM). This sensitivity is comparable to the level of taxol (0.25 μM) that inhibits Hela cell division (Schiff, et al. 1979. *Nature* 277:665-667).

Example 2: Isolation of β-tubulin cDNA sequences

Beta-tubulin cDNA sequences were determined for *P. microspora* Ne32, *P. ultimum,* and *P. cinnamomi* from RNA isolated from fungal mycelia. Automated dideoxynucleotide sequencing was performed by a contracting laboratory. Sequence comparison was performed using the BLAST program at the Internet site of the National Center for Biotechnology Information. The amino acid sequence alignment was performed using ClustalW program, and other analysis using MacVector program.

To isolate beta-tubulin cDNA sequences from *P. microspora* Ne32 and *P. ultimum,* four degenerate primers were designed according to conserved motifs in fungal beta-tubulin amino acid sequences. A forward degenerate primer BTUB1, 5'-CTGGGCYAAGGGYC AYTACACYGAG-3' (SEQ ID NO:7, was designed corresponding to amino acid residues Trp-Ala-Lys-Gly-His-Tyr-Thr-Glu (or WAKGHYTE in single letter amino acid code; SEQ ID NO:8); a reverse primer BTUB2, 5'-CGAAGAARTGRARNCGRGGGAARGG-3' (SEQ ID NO:9), corresponding to amino acid residues Pro-Phe-Pro-Arg-Leu-His-Phe-Phe (or PFPRLHFF in single letter amino acid code; SEQ ID NO:10); a forward primer BTUB3, 5'-CGAGCCYTACAACGCYACYCT-3' (SEQ ID NO:11), corresponding to amino acid residues Glu-Pro-Tyr-Asn-Ala-Thr-Leu (or EPYNATL in single letter amino acid code; SEQ ID NO:12); and a reverse primer BTUB4, 5'-CTCGTTCATGTTRSWCTCRGCCTC-3' (SEQ ID NO:13), corresponding to amino acid residues Glu-Ala-Glu-Ser-Asn-Met-Asn-Asp

(or EAESNMND in single letter amino acid code; SEQ ID NO:14). All primers were synthesized by a contracting laboratory according to our specifications.

In order to isolate beta-tubulin cDNA from *P. microspora* Ne32, five micrograms (5 μg) of total RNA from mycelia grown for 5 days was used to synthesize first-strand cDNA using primer BTUB4 in a 20 microliter (20 μl) reaction. One microliter (1 μl) of the cDNA product was used as template in Polymerase Chain Reactions (PCR). The cycling program contained 8 cycles of an annealing temperature of 52°C, followed by 22 cycles with an annealing temperature of 62°C. Degenerate primers BTUB3 and BTUB4 generated an amplification product of 0.8 kb, whereas BTUB2 and BTUB3 generated a product of 0.3 kb. The desired bands were gel-purified using Geneclean (BIO 101, Vista, CA), ligated into the pPCR2.1 vector (Invitrogen; San Diego, CA), and transformed into *E. coli* XL1 -Blue cells. Inserts were sequenced and used to synthesize a gene-specific forward primer NETUB5, 5'-GGGTGTCACCACTTGCTTGCGTTT-3' (SEQ ID NO:15), and a reverse primer NETUB6, 5'-TCGAGTTTCCGACGAAAGTGGACGA-3' (SEQ ID NO:16). To obtain full-length clones, a Marathon cDNA Library was constructed using one microgram (1 μg) mRNA according to manufacturer (Clontech; Palo Alto, CA). One microliter (1 μl) of this library was diluted 250-fold, and five microliters (5 μl) were used in Rapid Amplification of cDNA Ends (RACE) reactions using the PCR cycling program recommended by the manufacturer. For 5' RACE, library adaptor primer AP1 (Clontech) and primer NETUB6 generated a product of 1.3 kb. For 3' RACE, primers AP1 and NETUB5 generated a product of 1.0 kb. The desired bands were gel-purified and cloned into the pPCRII-TOPO vector (Invitrogen, Carlsbad, CA). The region between 1 to 1105 bp from the 5' RACE product was ligated at an internal *Bam*HI site with the region between 1106 to 1668 bp from the 3' RACE product to form the composite cDNA (Fig. 2). The resulting composite cDNA from *P. microspora* was 1668 bp long, designated as TUBB-pm, and its nucleotide and deduced amino acid sequence are shown in SEQ ID NO:1 and SEQ ID NO:2, respectively, as well as Fig 2. This cDNA encodes a protein of 446 amino acids with a calculated Mr of 49,832 and pI of 4.6. It contains 74 nucleotides in the 5' untranslated region (UTR), and 229 nucleotides in the 3' UTR followed by a 24 nucleotide poly (A) tail. A sequence AATAA (nucleotides 1539-1543 of SEQ ID NO:1) with the closest similarity to the animal and viral polyadenylation signal AATAAA (Proudfoot, N.J. and Brownlee, G.G. 1976. "3' Non-coding region

sequences in eukaryotic messenger RNA," *Nature* 263:211-214) was located 103 bp upstream of the poly (A) tract.

In order to isolate beta-tubulin cDNA from *P. ultimum,* five micrograms (5 μg) of total RNA from mycelia grown for six days was used to synthesize first strand cDNA with oligo-dT primer (GibcoBRL; Gaithersburg, MD) in a twenty microliter (20 μl) reaction. Two microliters (2 μl) of cDNA product were used as the template in PCR reactions with a cycling program similar to that described above. Degenerate primers BTUB1 and BTUB4 generated a product of 1.0 kb, whereas BTUB1 and BTUB2 amplified a product of 0.5 kb. The desired bands were gel-purified and ligated into the pPCR2.1 vector. Inserts were sequenced and used to design a gene-specific forward primer WT1L-U, 5'-CTAT CATGTGCACGTACTCGGTGTGC-3' (SEQ ID NO:17), and a reverse primer WT1L-L, 5'-CTGGGACGGTCAAAGCACGGTACTGC-3' (SEQ ID NO:18). For 5' RACE, first-strand cDNA was synthesized using primer WT1L-L and used as template in PCR reactions. Primers WT1L-L and Cap-Switch (Clontech) generated a 0.95 kb product using Advantage-GC cDNA PCR kit (Clontech). For 3' RACE, first strand cDNA was synthesized using CapFinder cDNA Library Construction kit (Clontech). With the resulting cDNAs as template, primers WTIL-U and CDS/3' (Clontech) generated two PCR products of 1.0 and 1.1 kb, respectively. PCR fragments were gel-purified and cloned into the pPCR2.1 vector. In *P. ultimum,* isolated tubulin cDNAs were of two types, one composite cDNA was 1650 bp long, and the other was 1537 bp long. These two cDNAs differ only in the position at which the poly (A) tail has been added. The region between 1 to 824 bp from the 5' RACE product was ligated at an internal *Mfe*I site with the region between 825 to 1650 bp from the 1.1 kb 3' RACE product to form the composite 1650 bp cDNA designated as TUBB-pu, and its nucleotide and deduced amino acid sequence are shown in SEQ ID NO:3 and SEQ ID NO:4, respectively, as well as Fig. 3. This cDNA encodes a protein of 446 amino acids with a calculated Mr of 50,047 and pI of 4.6. It contains 91 nucleotides in the 5' UTR, and 199 nucleotides in the 3' UTR followed by a 19 nucleotide poly (A) tract. Two imperfect polyadenylation signals were tentatively identified, ATATA at 57 bp upstream of poly (A) tract in the 1537 bp cDNA (nucleotides 1445-1449 of SEQ ID NO:3), and AATATT at 80 bp upstream of poly (A) tail in the 1650 bp cDNA (nucleotides 1546-1551 of SEQ ID NO:3). The sizes of these cDNAs match well with transcript sizes in Northern analysis as shown below, indicating they are complete.

Four gene-specific primers were synthesized based on the reported beta-tubulin cDNA sequence from *P. cinnamomi* (Weerakoon, et al. 1998. *Mycologia* 90:85-95). The forward primer PCBTUB1U (5'-CAGCGACAACATGAGAGAGCTCG-3'; SEQ ID NO:19) corresponds to region 270-292 in its cDNA sequence, the forward primer PCBTUB2U (5'-CGATGAGGTCATGTGCCTGGATAA-3'; SEQ ID NO:20) corresponds to region 867-890; the reverse primer PCBTUB3L (5'-AAACGGAGGCACGTGGTGATG-3'; SEQ ID NO:21) corresponds to region 984-1005; the reverse primer PCBTUB4L (5'-CGCGTC TATCTCATCCATTCCTCG-3'; SEQ ID NO:22) corresponds to region 1596-1619.

Five micrograms (5 μg) of total RNA from *P. cinnamomi* mycelia grown for 5 days was used to synthesize first-strand cDNA using oligo-dT primer in a 20 μl reaction. One microliter (1 μl) of the cDNA product was used as template for PCR. The cycling program comprised 30 cycles with an annealing temperature of 62°C. Primer PCBTUB1U and PCBTUB4L generated an amplification product of 1.3 kb, and primer PCBTUB2U and PCBTUB4L generated an amplification product of 0.75 kb. Primer PCBTUB1U or PCBTUB2U in combination with PCBTUB3L did not generate any product. The desired bands were gel-purified using Geneclean (BIO101), ligated into the pPCR2.1 vector (Invitrogen), and transformed into *E. coli* XL1-Blue cells. Four clones of the 1.3 kb fragment (clone # C16-1, 4, 9 and 10) and one clone of the 0. 75 kb fragment (clone # C10-5) were sequenced from both directions and found to conform to the same sequence. The nucleotide and deduced amino acid sequence of the 1.3 kb long cDNA, designated as TUBB-pc, are shown in SEQ ID NO:5 and SEQ ID NO:6, respectively, as well as Fig. 4. It encodes a 444 amino acid long beta- tubulin protein, with a calculated Mr of 50 kDa, and a pI of 4.7. There are 10 nucleotides in the 5' untranslated region (UTR), and 5 nucleotides in the 3' UTR.

A sequence encoding *P. cinnamomi* beta-tubulin has been previously reported (Weerakoon, et al. 1998. *Mycologia* 90:85-95; Genbank accession number U22050), and the deduced amino acid sequence of *P. cinnamomi* beta-tubulin disclosed herein was compared to that disclosed by Weerakoon et al. The TUBB-pc cDNA sequence shown in SEQ ID NO:5 and Fig. 4 differs by 36 nucleotides (2.7%) within the coding region from the one reported by Weerakoon et al. An alignment of the beta-tubulin amino acid sequences deduced from TUBB-pc (SEQ ID NO:6) and the one previously reported by Weerakoon

("U22050"; SEQ ID NO:23) is shown in Fig 5. The two sequences differ by 8 amino acids. Four are conserved changes, while the other four are nonconserved changes. One change is within each of the taxol-binding sites. In the taxol-binding region I (Amino Acids 1-31), Amino Acid V24 (valine at Amino Acid 24) in TUBB-pc differs from I24 (isoleucine at Amino Acid 24) in U22050. In the taxol-binding region II (Amino Acids 212-231), Amino Acid T219 (threonine at Amino Acid 219) in TUBB-pc differs from N219 (asparagine at Amino Acid 219) in U22050.

The deduced amino acid sequence of beta-tubulin from *P. microspora* (SEQ ID NO:2), *P. ultimum* (SEQ ID NO:4), and *P. cinnamomi* (SEQ ID NO:6) show features expected of beta-tubulin, as shown by an alignment with human β2-tubulin (SEQ ID NO:24) and from beta-tubulins from *Neurospora crassa* (SEQ ID NO:25), *A. nidulans* benA (SEQ ID NO:26), and *A. klebsiana* (SEQ ID NO: 27) depicted in Fig. 6. These sequences can be divided into N-terminal (Amino Acids 1-205), intermediate (Amino Acids 206-381) and C-terminal domains (Nogales, et al.1998. *Nature* 391:199-203). Their N-terminal domain contains conserved motifs important for GTP binding [Ala-Ile-Leu-Val-Asp-Leu-Glu-Pro-Gly-Thr-Met-Asp-Ser-Val-Arg or AILVDLEPGTMDSVR in single letter amino acid code (SEQ ID NO:28) and Ala-Val-Leu-Val-Asp-Leu-Glu-Pro-Gly-Thr-Met-Asp-Ser-Val-Arg or AVLVDLEPGTMDSVR in single letter amino acid code (SEQ ID NO:29) between Amino Acids 63-77 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 62-76 in SEQ ID NO:27], phosphate binding [Gly-Gly-Gly-Thr-Gly-Ser-Gly or GGGTGSG in single letter amino acid code (SEQ ID NO:30) between Amino Acids 140-146 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 139 and 145 in SEQ ID NO:27], and Mg^{2+} binding (Asp-Asn-Glu-Ala or DNEA in single letter amino acid code (SEQ ID NO:31) between Amino Acids 203-206 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 202-205 in SEQ ID NO:27) (Linse, K. and Mandelkow, E.M. 1988. "The GTP-binding peptide of β-tubulin: localization by direct photoaffinity labeling and comparison with nucleotide-binding proteins," *J Biol Chem* 263:15205-15210; and Farr, G.W. and Stemlicht, H. 1992. "Site-directed mutagenesis of the GTP-binding domain of β-tubulin," *J Mol Biol* 227:307-321). In addition, the N-terminal Met-Arg-Glu-Ile (or MREI

("U22050"; SEQ ID NO:23) is shown in Fig 5. The two sequences differ by 8 amino acids. Four are conserved changes, while the other four are nonconserved changes. One change is within each of the taxol-binding sites. In the taxol-binding region I (Amino Acids 1-31), Amino Acid V24 (valine at Amino Acid 24) in TUBB-pc differs from I24 (isoleucine at Amino Acid 24) in U22050. In the taxol-binding region II (Amino Acids 212-231), Amino Acid T219 (threonine at Amino Acid 219) in TUBB-pc differs from N219 (asparagine at Amino Acid 219) in U22050.

The deduced amino acid sequence of beta-tubulin from *P. microspora* (SEQ ID NO:2), *P. ultimum* (SEQ ID NO:4), and *P. cinnamomi* (SEQ ID NO:6) show features expected of beta-tubulin, as shown by an alignment with human β2-tubulin (SEQ ID NO:24) and from beta-tubulins from *Neurospora crassa* (SEQ ID NO:25), *A. nidulans* benA (SEQ ID NO:26), and *A. klebsiana* (SEQ ID NO: 27) depicted in Fig. 6. These sequences can be divided into N-terminal (Amino Acids 1-205), intermediate (Amino Acids 206-381) and C-terminal domains (Nogales, et al.1998. *Nature* 391:199-203). Their N-terminal domain contains conserved motifs important for GTP binding [Ala-Ile-Leu-Val-Asp-Leu-Glu-Pro-Gly-Thr-Met-Asp-Ser-Val-Arg or AILVDLEPGTMDSVR in single letter amino acid code (SEQ ID NO:28) and Ala-Val-Leu-Val-Asp-Leu-Glu-Pro-Gly-Thr-Met-Asp-Ser-Val-Arg or AVLVDLEPGTMDSVR in single letter amino acid code (SEQ ID NO:29) between Amino Acids 63-77 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 62-76 in SEQ ID NO:27], phosphate binding [Gly-Gly-Gly-Thr-Gly-Ser-Gly or GGGTGSG in single letter amino acid code (SEQ ID NO:30) between Amino Acids 140-146 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 139 and 145 in SEQ ID NO:27], and Mg^{2+} binding (Asp-Asn-Glu-Ala or DNEA in single letter amino acid code (SEQ ID NO:31) between Amino Acids 203-206 in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26, and between Amino Acids 202-205 in SEQ ID NO:27) (Linse, K. and Mandelkow, E.M. 1988. "The GTP-binding peptide of β-tubulin: localization by direct photoaffinity labeling and comparison with nucleotide-binding proteins," *J Biol Chem* 263:15205-15210; and Farr, G.W. and Stemlicht, H. 1992. "Site-directed mutagenesis of the GTP-binding domain of β-tubulin," *J Mol Biol* 227:307-321). In addition, the N-terminal Met-Arg-Glu-Ile (or MREI

in single letter amino acid code; (SEQ ID NO:32) motif has been shown to autoregulate the stability of beta-tubulin mRNA in animal cells (Yen, et al. 1988. "Autoregulated instability of β-tubulin mRNAs by recognition of the nascent amino terminus of β-tubulin," *Nature* 334:580-585). This motif and a variant Met-Arg-Glu-Leu (or MREL in single letter amino acid code; SEQ ID NO:33) are present in the fungal beta-tubulins shown in Fig. 6, and they may function similarly. The C-terminal domain has been reported to be important for interactions with microtubule-associated proteins (MAPS) and motor proteins (Nogales, et al. 1998 Nature 391:199-203). Sequences near the C-terminus are hypervariable and acidic, a common feature of beta-tubulins (Sullivan, K.F. 1988. "Structure and utilization of tubulin isotypes," *Ann Rev Cell Biol* 4:687-716).

The amino acid sequence of beta-tubulins from different organisms are well conserved and exhibit at least 63% identity (Oakley, B.R. 1994. "Gamma-tubulin." In: Hyams JS, Lloyd CW (eds) *Microtubules.* Wiley-Liss, New York, pp. 38-45). Table I shows the percentage identity between the beta-tubulin amino acid sequence of *P. microspora* and *P. ultimum* with beta-tubulins of other organisms. The beta-tubulin from *P. microspora* shares the highest identity (93-97%) with filamentous ascomycetes such as *A. flavus, A. nidulans* benA and *N. crassa,* and lower identity (73-78%) with single-cell ascomycetes and oomycetes. Its identity (78-85%) with beta-tubulin from non-fungal organisms is also relatively low. In contrast, beta-tubulin from *P. ultimum* shows the highest identity (96-97%) with beta-tubulin from two oomycetes, *A. klebsiana* and *P. cinnamomi,* but shares limited identity (71-78%) with beta-tubulin from ascomycetes. The beta-tubulin from *P. ultimum* also shows relatively high identity (86-93%) with beta-tubulin from non-fungal organisms such as the green algae *C. reinhardtii,* the protozoa *T. thermophila,* the slime mold *Physarum polycephalum*, and various animals.

Example 3: Extraction of genomic DNA and Southern analysis

Since multiplicity within the genes that encode beta-tubulin could affect the taxol-dependent property of microtubules, Southern analysis was used to determine whether *P. microspora* Ne32 and *P. ultimum* harbor one or more copies of beta-tubulin gene.

Mycelia of *P. microspora* Ne32 or *P. ultimum* grown for three days were harvested, and genomic DNA was isolated using Elu-Quik Hi-Volume Genomic kit (Schleicher

Table I: Amino Acid Sequence Identity Between Beta-tubulin from P. *microspora* or *P. ultimum* and Other Organisms[a]

Organism	Accession No.	Percentage of Identical Amino Acids	
		P. Microspora	*P. ultimum*
Saccharomyces cerevisiae	VO 1296	73	71
Schizosaccharomyces pombe	M10347	78	76
Aspergillus flavus	M38265	94	77
Aspergillus nidulans benA	M17519	93	77
Neurospora crassa	A25377	97	78
Achlya klebsiana	P20802	78	97
Phytophthora cinnamomi	U22050	77	96
Chlamydomonus reinhardtii (β1 and β2)	P04690	78	91
Tetrahymena thermophila (β1 and β2)	P41352	80	93
Physarum polycephalum (slime mold) (β1)	P07436	81	90
Drosophila melanogaster (fruit fly) (β1)	M20419	83	86
Xenopus laevis (β4)	P30883	85	89
chicken (β2)	P32882	84	88
mouse (β3)	C25437	84	89
human (β2)	P05217	84	89

[a] The amino acid sequences of beta-tubulin were retrieved from Genbank or Swiss-Prot. Pairwise identity was performed either with ClustalW or BLAST program.

Schuelle, Neene, NH). Five micrograms (5 μg) of genomic DNA was digested with restriction enzymes for 4 hours at 37°C, separated on 0.8% agarose gel, transferred to Nylon filters (Tropix, Bedford, MA), and wet filters were cross-linked using GS Gene Linker UV Chamber (Biorad; Hercules, CA). Southern blotting was performed under stringent conditions according to standard protocols (Sambrook et al. 1989. *Molecular Cloning: A*

their microtubules, we examined the ability of these fungal cells to specifically bind to [^{3}H]taxol. The total binding of [^{3}H]taxol to fungal cells consists of specific binding to microtubules and nonspecific binding to other cellular structures. The values of total and nonspecific binding were determined by binding of [^{3}H]taxol to fungal cells in the absence or presence of 100-fold excess unlabeled taxol. The specific binding of [^{3}H]taxol was then calculated as the difference between the amount of total and nonspecific binding.

Fresh mycelia from *P. microspora* Ne32, and *P. ultimum* were grown in 140 milliliters modified M1D media in Roux bottles at 24°C for 1-2 days. These actively growing mycelia were transferred to 50 milliliter conical tubes, and centrifuged at 7,000 rpm for 5 minutes at room temperature. Mycelia were suspended in 1 milliliter remaining M1D medium and 1 milliliter fresh M1D medium. Cells were either untreated or pretreated with the anti-mitotic drug thiabendazole to depolymerize microtubules. In pretreated cells, thiabendazole (in DMSO) was added to desired concentrations, and DMSO was adjusted to the same concentration in all samples. Samples were then incubated at room temperature for 3 hours. Subsequently, [^{3}H]taxol (3.7 X 10^7 Bq/ml, Moravek) was added to desired concentrations either in the presence or absence of 100-fold excess unlabeled taxol. Samples were incubated for 2 hours at room temperature, then quenched on ice. [^{3}H]taxol binding to *P. microspora* cells was performed in the presence of 0.1% (v/v) Triton X-100 to disrupt the cell membrane.

Each GFC filter (Whatman; Clifton, NJ) was weighed using an analytical balance. For *P. ultimum*, mycelia were transferred from conical tubes onto GFC filter hold by a 3-piece filter funnel (Whatman). Conical tubes were washed three times with 10 milliliters of MilliQ H_2O (Millipore, Inc.; Bedford, MA). Mycelia on GFC filter were washed with 120 milliliters of MilliQ H_2O. For *P. microspora*, mycelia were collected by centrifugation at 5,000 rpm for 5 minutes at room temperature, and washed three times with 40 milliliters of MilliQ H_2O. Mycelia were transferred onto GFC filter, and combined with residual mycelia after rinsing conical tubes with 5 milliliters of MilliQ H_2O. GFC filters were dried at 80°C in an oven overnight and then weighed to obtain mycelia dry weight. Filters were counted for 5 minutes under 20 milliliters of Cytoscint (Fisher Scientific; Pittsburgh, PA) in a Beckman LS3801 scintillation counter. Specific binding was calculated as the difference between [^{3}H]taxol bound in the presence and absence of a 100-fold excess unlabeled taxol.

Nonspecific binding was determined as binding in the presence of 100-fold excess unlabeled taxol.

[^{3}H]taxol was found to bind specifically to *P. ultimum* cells, and the amount of specific binding increased as a function of [^{3}H]taxol concentration (Fig. 7A). In addition, in cells pretreated with thiabendazole to reduce the amount of assembled microtubules, the specific binding of [^{3}H]taxol decreased in a dose-dependent manner (Fig. 7B). In fact, treatment with 1 mM of thiabendazole completely abolished the specific binding of [^{3}H]taxol. These results indicate that taxol is able to interact with *P. ultimum* microtubules, and are consistent with the fact that this organism is sensitive to taxol.

On the other hand, initial experiments showed very low amount of specific binding of [^{3}H]taxol to *P. microspora* (data not shown). This result could be due to inefficient interactions between taxol and *P. microspora* microtubules, or alternatively due to a membrane barrier which prevents intracellular accumulation of [^{3}H]taxol. In animal cells, taxol crosses the cell membrane by diffusion due to its hydrophobic character (Manfredi et al. 1982. *J Cell Biol* 94:688-696). In some cases, resistance to taxol has been associated with P-glycoprotein, a membrane-located pump which causes drug efflux (Jachez et al. 1993. "Restoration of taxol sensitivity of multidrug-resistant cells by the cyclosporine SDZ PSC 833 and the cyclopeptide SDZ 280-446," *J Natl Cancer Inst* 85:478-483)). There is no information available as to whether *P. microspora* has such system. It has been shown that treatment of animal cells with nonionic detergents such as 0.1% (v/v) NP-40 or Triton X-100 disrupt the cell membrane, release most soluble proteins including unassembled tubulins, but leave assembled microtubules intact (Schliwa et al. 1981. "Stabilization of the cytoplasmic ground substance in detergent-opened cells and a structural and biochemical analysis of its composition," *Proc Natl Acad Sci USA* 78:4329-4333; Duerr et al. 1981. "Molecular analysis of cytoplasmic microtubules in situ: identification of both widespread and specific proteins," *Cell* 24:203-222; Manfredi et al. 1982. *J Cell Biol* 94:688-696). To evaluate whether a membrane barrier was responsible for the low specific binding, [^{3}H]taxol binding to *P. microspora* cells in the presence of Triton X-100 was performed. *P. microspora* cells treated with Triton X-100 (0.1% (v/v)) showed none or very little specific binding of [^{3}H]taxol up to 75 nM [^{3}H]taxol (Fig, 7A). Furthermore, cells pretreated with thiabendazole also showed no specific binding of [^{3}H]taxol in the presence of Triton X-100

(data not shown). These results indicate that taxol is unable to interact or interacts poorly with microtubules of *P. microspora*, and are consistent with the fact that this organism is resistant to taxol. In summary, the [^{3}H]taxol binding results demonstrate that the properties of β-tubulin in these organisms determine their differential sensitivity to taxol.

Taxol stabilizes MTs by binding to beta-tubulin in assembled MTs, and its binding site has been characterized by photo cross-linking, electron crystallography, and mutagenesis. Regions between Amino Acids 1-31 and 217-231 were found to cross-link to the C-3' and C-2 group of taxol, respectively (Rao, et al. 1994. *J Biol Chem* 269:3132-3134; and Rao, et al. 1995. *J Biol Chem* 270:20235-20238). Recently, the structure of the beta-tubulin dimer was solved by electron crystallography of zinc induced sheets of tubulin dimer (Nogales, et al.1998. *Nature* 391:199-203). Modeling of taxol bound to this structure shows that the C-3' group of taxol is near Amino Acids 15-25 of beta-tubulin (near the top of helix H1), and the C-2 group is near Amino Acids 212-222 (near helix H6 and the loop between H6-H7). The identification of Amino Acids 15-25 and 217-222 in both cross-linking and electron crystallography studies indicate these regions are important for taxol binding. In addition, the electron crystallography model also shows that Leu273 of bovine beta-tubulin (located in the M-loop) contacts the taxane ring of taxol (Nogales, et al.1998. *Nature* 391:199-203). In addition, mutations at Phe270 or Ala364 in the M40 isotype of beta-tubulin result in taxol resistance in human ovarian cells (Giannakakou, et al. 1997. *J Biol Chem* 272:17118-17125).

Since the Amino Acids 270, 273 and 364 (marked by # in Fig. 6) do not differ among the fungal beta-tubulins listed in Fig. 6, they are not responsible for the differential taxol response among these organisms. However, comparison of Amino Acids 1-31 and 212-231 (defined here as taxol binding region I and II, respectively) from beta-tubulins of organisms that are taxol-resistant or taxol-sensitive reveal residues that are important for taxol interaction. Fig. 8 provides a comparison of the taxol binding region I and taxol binding region II amino acid sequences for pig (I, SEQ ID NO:34; II, SEQ ID NO:35), human β2 (I, SEQ ID NO:36; II, SEQ ID NO:37), *Drosophila* β1 (I, SEQ ID NO:38; II, SEQ ID NO:39), *Xenopus* β4 (I, SEQ ID NO:40; II, SEQ ID NO:41), *Tetrahymena* (I, SEQ ID NO:42; II, SEQ ID NO:43), *Physarum* β1 (I, SEQ ID NO:44; II, SEQ ID NO:45), *P. ultimum* (I, SEQ ID NO:46; II, SEQ ID NO:47), *P. cinnamomi* (I, SEQ ID NO: 48; II, SEQ

ID NO: 49) *A. klebsiana*(I, SEQ ID NO:50; II, SEQ ID NO:51), *P. microspora* (I, SEQ ID NO:52; II, SEQ ID NO:53), *A. nidulans* benA (I, SEQ ID NO:54; II, SEQ ID NO:55), and *S. cerevisiae* (I, SEQ ID NO:56; II, SEQ ID NO:57).

Beta-tubulins from taxol-sensitive organisms such as human, pig, *Drosophila, Xenopus, Tetrahymena* and *Physarum* are highly conserved in taxol binding region I and II, and are identical between Amino Acids 15-25 and 217-222 (except a conserved substitution at Amino Acid 23 in *Drosophila* β1). Beta-tubulin from *P. ultimum* displays only four substitutions compared to the above sequences, none of which occurs between Amino Acids 15-25 and 217-222. This similarity is consistent with the fact that *P. ultimum,* like the animal organisms noted above, is taxol-sensitive. Also consistent with this, previous biochemical studies of animal tubulins and data of [^{3}H]taxol binding to *P. ultimum* demonstrated herein (Fig. 7A and 7B), show that taxol binds beta-tubulin in assembled MTs of these organisms (Kellogg, et al. 1989. *J Cell Biol* 109:2977-2991; and Manfredi, J.J. and Horwitz, S.B. 1984. *Pharmacol Ther* 25:83-125). Beta-tubulin sequences from *P. ultimum* and *A. klebsiana* are identical in taxol binding region I and II except Amino Acid 219, but *A. klebsiana* is relatively resistant to taxol (IC_{50} > 11.7 μM). This reduced sensitivity is due in part to the fact that *A. klebsiana* contains an asparagine at Amino Acid 219, whereas *P. ultimum,* and six other beta-tubulins from taxol-sensitive organisms, have threonine.

Beta-tubulins from taxol-resistant organisms such as *P. microspora, A. nidulans* and *S. cerevisiae* are similar to each other within taxol binding region I and II, but differ from the above discussed sequences in seven positions (19, 22, 23, 25, 218, 219, and 221) within regions 15-25 and 217-222. The [^{3}H]taxol binding data presented herein (Fig. 7A and 7B), together with previous biochemical studies (Yoon, Y. and Oakley, B.R. 1995. *Biochem* 34:6373-6381; and Bames, et al. 1992. *Mol Biol Cell* 3:29-47), show that beta-tubulins in assembled MTs of these organisms are unable to efficiently bind taxol. These sequences contain the asparagine (or glutamine in the case of *S. cerevisiae)* at Amino Acid 219, as observed in *A. klebsiana,* a substitution that contributes in part to the reduced sensitivity to taxol in these fungi. Other substitutions, including which involve differences in charge and polarity such as changes from Lys19 to Ala, Glu22 to Gln, and Val23 to Thr, also contribute to the taxol resistant phenotype of these organisms.

These results indicate that a number of residues including threonine at Amino Acid 219 are important in the binding of beta-tubulin to taxol. In particular, Amino Acid 219 plays an important role in determining taxol binding property of beta-tubulin and, consequently, the taxol-sensitivity of cells. Beta-tubulins from taxol-sensitive species have Thr219 (threonine at Amino Acid 219), while those from taxol-resistant species have Asn219 (asparagine at Amino Acid 219) or Glu219 (glutamine at Amino Acid 219). The taxol sensitivity of *P. cinnamomi* is consistent with the presence of Thr219 in TUBB-pc (SEQ ID NO:6) and not Asn219 as previously reported by Weerakoon et al. The presence of Asn219 (asparagine at Amino Acid 219) found in *P. microspora* is consistent with the taxol resistance of this species. Using the information that the presence of threonine at Amino Acid 219 in beta-tubulins corresponds to taxol-binding and taxol-sensitivity, taxol analogs or other compounds can be designed which mimic the interaction of taxol with beta-tubulin. Further, such information can also be used to generate mutant beta-tubulins resistant to taxol by substituting the threonine for another amino acid residue at Amino Acid 219.

Example 6: Sensitivity to microtubule-depolymerization drugs.

The effect of several MT-depolymerization drugs on the growth of *P. microspora* Ne32, *P. ultimum* and *A. klebsiana* was examined. These drugs included colchicine, colcemid (a synthetic derivative of colchicine), and two benzimidazole drugs, nocodazole and thiabendazole.

Colchicine, colcemid, nocodazole, and thiabendazole were obtained from Sigma Chemical Company (St. Louis, MO). A stock solution of colchicine was prepared in water, and other stock solutions in DMSO. An agar plug (6 mm in diameter) of fresh mycelia was transferred onto PDA plates containing 1 % (v/v) DMSO in the presence or absence of an anti-microtubule agent. Fungal colonies were grown at 24°C for 24 hours in the case of *P. ultimum* or 48 hours in the case of *P. microspora* and *A. klebsiana*. The growth inhibitory effect of these anti-mitotic agents was measured by the size of colony diameters.

Biochemical and genetic evidence has shown that these drugs bind to beta-tubulin in the tubulin dimer and cause MT depolymerization (Davidse, L.C. and Flach, W. 1978. "Interaction of thiabendazole with fungal tubulin," *Biochim Biophys Acta* 543:82-90; Jung,

M.K. and Oakley, B.R. 1990. "Identification of an amino acid substitution in the β-tubulin gene of *Aspergillus nidulans* that confers thiabendazole resistance and benomyl supersensitivity," *Cell Motil Cytoskeleton* 11:87-94; Manfredi, J.J. and Horwitz, S.B. 1984. *Pharmacol Ther* 25:83-125). It has been shown that many fungi are resistant to colchicine (Cameron et al. 1990. *J Biol Chem* 265:15245-15252; Kilmartin, J.V. 1981. "Purification of yeast tubulin by self-assembly *in vitro*," *Biochem* 20:3629-3633; and Davidse, L.C. and Flach, W. 1977. "Differential binding of methyl benzimidazole-2-yl-carbarnate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of *Aspergillus nidulans*," *J Cell Biol* 72:174-193), but are sensitive to nocodazole (Kilmartin, J.V. 1981. *Biochem* 20:3629-3633) and thiabendazole (Davidse, L.C. and Flach, W. 1978. *Biochim Biophys Acta* 543:82-90).

As shown in Table II, the three fungal species tested herein were resistant to colchicine and colcemid (IC_{50} >100 μM), and were sensitive to nocodazole (IC_{50} 2-22 μM). These results are consistent with the studies noted above. In contrast, these fungi were differentially sensitive to thiabendazole. *P. microspora* was highly sensitive (IC_{50} 3 μM), while *P. ultimum* and *A. klebsiana* were less sensitive (IC_{50} 270-350 pM). These results demonstrate that the biochemical properties of beta-tubulin differ in these three fungi.

Table II: Sensitivity of fungi to microtubule depolymerization drugs

Drug	*P. microspora*	*P. ultimum*	*A. klebsiana*
colchicine	>100	>100	>100
colcemid	>100	>100	>100
nocodazole	2	22	2
thiabendazole	3	350	270

Mutations at Amino Acids 6, 165, 167, 198, 200 and 241 in beta-tubulin (marked by asterisks in Fig. 6) result in altered sensitivity to thiabendazole and other benzimidazole drugs in yeast, *N. crassa, A. nidulans* benA, and *Trichoderma viride* (Thomas, et al. 1985. "Isolation and characterization of mutations in the β-tubulin gene of *Saccharomyces cerevisiae*," *Genetics* 112:715-734; Orbach, et al. 1986. *Mol Cell Biol* 6:2452-2461; Jung, et

al. 1992. "Amino acid alterations in the β-tubulin gene of *Aspergillus nidulans* that confer benomyl resistance," *Cell Motil Cytoskeleton* 22:170-174; Jung, M.K. and Oakley, B.R. 1990. *Cell Motil Cytoskeleton* 17:87-94; Fugimura, et al. 1992. "A single amino-acid substitution in the beta-tubulin gene of *Neurospora* confers both cabendazim resistance and diethofencarb sensitivity," *Curr Genet* 21:399-404; and Goldman et al. 1993. *Mol Gen Genet* 240:73-80). These six residues in beta-tubulin from *P. microspora* are identical to those observed in beta-tubulin from other thiabendazole sensitive species such as *N. crassa* and *A. nidulans* benA. In contrast, beta-tubulin from *P. ultimum* and A. *klebsiana* differ at Amino Acids 165, 167 and 200. It has been previously shown that a phenylalanine-to-tyrosine change at Amino Acid 167 results in benzimidazole resistance in *N. crassa* (Orbach, et al. 1986. *Mol Cell Biol* 6:2452-2461), and the fact that both *P. ultimum* and *A. klebsiana* have a tyrosine at this position accounts for their resistance to such drugs. In summary, the differential sensitivity to thiabendazole exhibited by these three fungi is consistent with the comparison of fungal beta-tubulins shown in Fig. 6.

Example 7: Production of Antibodies Capable of Distinguishing Taxol-Binding and Non-Binding Beta-tubulins

Monoclonal or polyclonal antibodies can be raised against the following antigens: 1) native beta-tubulins extracted from *P. microspora, P. ultimum*, or *P. cinnamomi*; 2) beta-tubulins of *P. microspora, P. ultimum*, or *P. cinnamomi* produced from a heterologous system such as *E. coli*, yeast, and insect cells; and 3) synthetic peptide corresponding to the SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, and preferably comprising at least one taxol binding region. The antibodies are used to interact with the above mentioned beta-tubulins using Elisa or Western blotting using standard protocols (Harlow, E.D. and Lane, D. 1988. *Antibodies: A Laboratory Manual*). The antibodies which could distinguish the taxol binding beta-tubulin from the taxol non-binding beta-tubulin are selected as the reagent.

A specific example is to raise polyclonal or monoclonal antibodies to synthetic peptides corresponding to SEQ ID NO:4 or SEQ ID NO:6 which comprise at least one taxol binding region, for instance containing the taxol-binding region II comprising Thr219 or in which the Thr219 is replaced by Asn219/Gln219. The ability of these antibodies to interact with beta-tubulin is examined using Elisa using standard protocols. The antibody which can binds to peptide containing Thr219 but not to peptide containing Asn/Gln 219 is selected as

the reagent which is specific for the taxol-binding site containing Thr 219. On the other hand, the antibody which specifically binds to the peptide containing Asn219/Gln 219 but not to the peptide containing Thr 219 is selected as the reagent which specifically recognizes taxol binding site devoid of Thr 219.

Example 8: Screening Assays to Detect Beta-Tubulin in Matter

Several assays can be used to determine if a composition of matter contains beta-tubulin capable of binding taxol. These assays are useful for screening a variety of compositions of matter, including living matter such as plants or microorganisms, or non-living matter such as plant materials or patient samples for the presence of beta-tubulin.

The first assay is performed using Northern or Southern hybridization method well known in the art (Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). The total RNA, mRNA or genomic DNA are isolated from the composition of matter and separated by electrophoresis. DNA, synthetic oligonucleotide, or RNA corresponding to the coding region or a portion of beta-tubulin (e.g., derived from SEQ ID NO:1, SEQ ID NO: 3 or SEQ ID NO:5) which comprises at least one taxol binding region will be used to synthesize isotopically labeled probes. Hybridization with a probe derived from SEQ ID NO:1 will indicate beta-tubulin with high probability of taxol resistance. On the other hand, the hybridization with a probe derived from SEQ ID NO:3 or SEQ ID NO:5 will indicate beta-tubulin with a high probability of taxol sensitivity.

The second assay is to use a PCR-based assay using standard protocols (Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). Both genomic DNA or cDNA converted from total RNA or mRNA are used as template in a PCR assay. Gene-specific or degenerate primers corresponding to the coding region of beta-tubulin (e.g., derived from SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5) which comprises at least one taxol binding region will be synthesized. Only DNA containing the appropriate primer sequences will be amplified, and all other variations will be suppressed. The amplification of PCR fragment of the predicted size using primers derived from SEQ ID NO:3 or SEQ ID NO:5 but not from primers derived from SEQ ID NO:1 will indicate high probability of taxol binding beta-tubulin. On

the other hand, the amplification of a PCR fragment of the predicted size using primers derived from SEQ ID NO:1 but not from primers derived from SEQ ID NO:3 or SEQ ID NO:5 will indicate high probability of taxol non-binding beta-tubulin. The subsequent obtaining of the beta-tubulin sequence and examination of the presence or absence of Thr219 residue will provide further determination.

The third assay is to use Elisa or Western blotting using standard protocols (Harlow, E.D. and Lane, D. 1988. *Antibodies: A Laboratory Manual*). Cell extracts of the composition of matter are prepared. Synthetic peptide, or native beta-tubulins extracted from *P. microspora, P. ultimum*, or *P. cinnamomi*, or produced from a heterologous system such as *E. coli*, yeast, and insect cells will be used to raise polyclonal or monoclonal antibodies. The antibodies will be used in the above mentioned Elisa or Western blotting. The antibody which recognizes the taxol binding from the non taxol binding is used in these assays.

Example 9: Construction of Taxol-sensitive and Taxol-resistant Isogenic Strains

P. ultimum contains a single beta-tubulin. In vitro, its beta-tubulin gene or cDNA can be altered to change the Thr219 to a different residue, for instance to Asn219 or Gln219. This altered DNA sequence is cloned into a transformation vector, and used to transform the wild-type strain *P. ultimum* using established protocols (Balance, et al. 1985. *Gene* 36:321-331). Homologous recombination between the wild-type beta-tubulin gene and the modified beta-tubulin in the vector occur. Transformed fungus are selected on media containing taxol. The taxol-resistant clones are selected and their beta-tubulin cDNA sequenced to confirm the absence of Thr 219. The taxol-resistant isogenic strain of *P. cinnamomi* is similarly constructed and used in screening assays as described in later examples. The only difference between these isogenic strains is that the taxol-sensitive strain is capable of binding to taxol due to the presence of Thr 219, and the taxol-resistant strain is incapable of binding to taxol due to the absence of Thr 219. Such taxol-resistant strains can be used in combination with the wild-type taxol-sensitive strains for screening as described in later examples.

Example 10: Screening Assays to Detect Taxol or Taxol-like Compounds in Matter

Several assays can be used to detect taxol or taxol-like compounds in a composition of matter. These assays are useful for screening a variety of compositions of matter, including living matter such as plants or microorganisms, or non-living matter such as plant materials, patient samples, or compound libraries for the presence of taxol or taxol-like compounds.

One screening method is to use taxol-resistant *P. microspora* in combination with the taxol-sensitive *P. ultimum* or *P. cinnamomi*. Taxol inhibits the growth of both *P. ultimum* by binding to their beta-tubulin, while taxol does not affect the growth of *P. microspora* since it does not interact with its beta-tubulin. A composition of matter which is capable of the inhibition of *P. ultimum*, but not *P. microspora* has a high probability of containing taxol-or a taxol-like compound.

An improved screening method uses taxol-sensitive and taxol-resistant isogenic strains of *P. ultimum* or *P. cinnamomi* as described in above example. The composition of matter is used to examine its effect on the growth of both the taxol-sensitive as well as the taxol-resistant strains. The inhibition of the taxol-sensitive strain but not the taxol-resistant strain indicates the presence of taxol or a taxol-like compound. On the other hand, the non-inhibition of both the taxol-sensitive and taxol-resistant strains indicates the absence of taxol or a taxol-like compound.

Composition of matter can be screened for the presence of taxol or taxol-like compounds based on their ability to promote the assembly of microtubules, as well as to stabilize assembled microtubules in conditions such as cold which otherwise cause depolymerization (Schiff, et al. 1979; Horwitz, 1981). The alpha- and beta-tubulins used in these assays can be from the following sources. 1) native microtubules consisting of beta-tubulins and alpha-tubulins extracted from *P. ultimum* or *P. cinnamomi*; 2) beta-tubulins extracted from *P. ultimum* or *P. cinnamomi* and interacted with another source of alpha-tubulin, for example, bovine alpha-tubulin; 3) all or portions of SEQ ID NO:4 or SEQ ID NO:6 produced from a heterologous system such as *E. coli*, yeast, insect cells or the like and alpha-tubulin either from *P. ultimum, P. cinnamomi* or another source. If the composition matter has the ability to promote the assembly of these MTs, as well as to prevent

depolymerization of assembled MTs in conditions which otherwise cause depolymerization, the composition of matter is likely to contain taxol or a taxol-like compound. Meanwhile, these isolated compounds should be unable to promote the assembly of MTs as well as prevent the depolymerization of MTs which consist of beta-tubulin derived from *P. microspora*.

An alternative screening method can be performed based on the competitive inhibition of [^{3}H]taxol binding to MTs in *P. ultimum* or *P. cinnamomi* by taxol or taxol-like compounds. The specific binding of [^{3}H]taxol to *P. ultimum* is performed as described in Example 5. The amount of [^{3}H]taxol specifically bound to *P. ultimum* in the absence of inhibitors is considered 100%. The composition of matter is added to the assay mixture, and the amount of [^{3}H]taxol specifically bound to *P. ultimum* in the presence of the composition of matter is measured. Reduction in the [^{3}H]taxol specific binding indicates that the composition of matter possesses taxol-like quality. If increased concentrations of the composition of matter can completely inhibit the [^{3}H]taxol binding, it will indicate that the compound likely binds to the same binding site in the beta-tubulin in MTs.

The screening of compositions of matter for taxol or taxol-like compounds can be performed by one of the above methods. Preferably, one of the first two methods is used for an initial screening, since they are simple to perform and easily handle large amounts of samples. The third and fourth method can be used for subsequent screening.

Example 11: Screening Assay to Distinguish Taxol-Sensitivity of A Patient Sample

In this diagonostic assay, antibodies depicted in Example 7 which could distinguish taxol-binding beta-tubulin from the non-binding beta-tubulin are used. Cellular proteins are extracted from a tumor specimen from a patient sample to detect the presence of a beta-tubulin with either taxol-binding or non-binding capabilities.

For example, in a diagnostic assay to screen for tumors resistant to taxol, the taxol binding regions of taxol-sensitive and taxol-resistant beta-tubulins of the present invention (e.g., SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6) are used to raise monoclonal or polyclonal antibodies using standard methods well known in the art (Harlow, E.D. and Lane, D. 1988. *Antibodies: A Laboratory Manual).* For example, monoclonal antibody probes are

reacted with a patient sample, such as a tumor specimen, to detect the presence of a beta-tubulins with either taxol-binding or non-binding capabilities. Visualization of antibody-antigen binding is mediated through any means known in the art, e.g., secondary radiolabeled or fluorescent antibodies or colorimetric methods using peroxidase and/or alkaline phosphatase (Harlow, E.D. and Lane, D. 1988. *Antibodies: A Laboratory Manual*). The detection of beta-tubulins with taxol-binding capability, i.e., taxol-sensitive beta-tubulins, corresponds to a positive response to taxol therapy. Alternatively, the detection of non-binding taxol-resistant beta-tubulins and/or the absence of taxol-sensitive beta-tubulins corresponds to a diminished or lack of response to taxol therapy.

Example 12: Biocontrol of Taxol-sensitive Pathogenic Oomycetes Using *P. microspora* on Plants

Many oomycetes including *P. ultimum* and *P. cinnamomi* are plant pathogens which can cause crop damage and result in severe economical loss. For instance, *P. ultimum* causes root rot of beans, and *P. cinnamomi* causes root rot of Avacado (ATCC: Catalogue of Filamentous Fungi, 18th edition, 1991). Many of the oomycetes are also taxol-sensitive (Young, et al. 1992. "Antifungal properties of taxol and various analogues," *Experientia* 48:882-885). Two of these strains, *P. ultimum* and *P. cinnamomi,* contain threonine at Amino Acid 219.

The biocontrol method of the present invention involves a two-step process: 1) the taxol sensitivity of the plant pathogen is determined and 2) if the plant pathogen is taxol-sensitive, a taxol-producing *P. microspora* is applied to the infected plants and surrounding soil as a source of growth-inhibiting taxol.

The taxol sensitivity of the plant pathogen is first determined. One method of identifying taxol sensitivity is to determine the presence or absence of threonine at Amino Acid 219. If the identity of the pathogen is known, DNA and protein databases are searched to determine whether the beta-tubulin sequence has been reported, if so, the identity of Amino Acid 219 is determined from the database. If the pathogen's beta-tubulin sequence is unavailable, the cDNA sequence is isolated and analyzed to determine the identity of Amino Acid 219. The presence of threonine at Amino Acid 219 in the pathogen's beta-tubulin gene indicates sensitivity to taxol, and thus, the pathogen is designated as treatable by a taxol-

producing *P. microspora*. If Amino Acid 219 is not threonine, the taxol sensitivity would have to be determined by other means such as taxol growth inhibition. Other screening methods presented herein for determining the presence of taxol-binding beta-tubulins can also be used.

It has been previously reported that *P. microspora* produces taxol at 50 ug /liter (Strobel, et al. 1996. *Microbiol* 142:435-440) and secrets taxol outside of the fungal cell. At this taxol concentration, the growth of *P. ultimum* and *P. cinnamomi* is inhibited (see Fig. 1). For treatment, *P. microspora* is inoculated to the area of plants or soil infected with the taxol-sensitive pathogen. The growth of *P. microspora* results in the secretion of taxol, which consequently inhibits the growth of these taxol-sensitive organisms.

Example 13: Use of Crystal Structures in Design of Antineoplastic or Antifungal Drugs

The three-dimensional structure of beta-tubulins are used to rationally design taxol-like compounds using methods known in the art (Ealick, et al. 1991. "Application of crystallographic and modeling methods in the design of purine nucleoside phosphorylase inhibitors," *Science* 88:11540-11544; Rossman, et al. 1991. "Application of crystallography to the design of antiviral agents," *Infectious Agents and Disease* 1:3-10). As taught by the present invention, application of the knowledge that Thr219 in the protein structure plays an important role in binding of taxol to taxol-like compounds can be critically applied to the development of drugs having taxol-like activities.

We claim:

1. A purified DNA segment encoding a beta-tubulin of the fungal species *Pestalotiopsis microspora* or a portion thereof.

2. The DNA segment of Claim 1, wherein said portion encodes at least one taxol binding site.

3. The DNA segment of Claim 2, wherein said portion encodes a protein having taxol binding site I and taxol binding site II.

4. The DNA segment of Claim 3, wherein said protein is able to interact with alpha-tubulin.

5. The DNA segment of Claim 1, wherein said DNA segment comprises at least a portion of SEQ ID NO:1.

6. The DNA segment of Claim 5, wherein said portion comprises the nucleotide sequence from nucleotide 75 through nucleotide 167 of SEQ ID NO:1.

7. The DNA segment of Claim 6, wherein at least one nucleotide in said nucleotide sequence is substituted.

8. The DNA segment of Claim 5, wherein said portion comprises the nucleotide sequence from nucleotide 708 through nucleotide 764 of SEQ ID NO:1

9. The DNA segment of Claim 8, wherein at least one nucleotide in said nucleotide sequence is substituted.

10. The DNA segment of Claim 9, wherein nucleotide 729, nucleotide 730, nucleotide 731 or mixtures thereof are substituted.

11. The DNA segment of Claim 5, comprising the nucleotide sequence from nucleotide 75 to nucleotide 1412 of SEQ ID NO:1, said DNA segment encoding a beta-tubulin.

12. The DNA segment of Claim 11, wherein at least one nucleotide in said nucleotide sequence is substituted and wherein the taxol binding capacity of said beta-tubulin is not altered.

13. The DNA segment of Claim 11, wherein at least one nucleotide in said nucleotide sequence is substituted and wherein the taxol binding capacity of said beta-tubulin is altered.

14. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pestalotiopsis microspora*.

15. The amino acid sequence of Claim 14, wherein said portion comprises at least one taxol binding site.

16. The amino acid sequence of Claim 15, wherein said portion comprises taxol binding site I and taxol binding site II.

17. The amino acid sequence of Claim 16, wherein said portion is able to interact with alpha-tubulin.

18. The amino acid sequence of Claim 14, wherein said amino acid sequence comprises at least a portion of the beta-tubulin as depicted in SEQ ID NO:2.

19. The amino acid sequence of Claim 18, wherein said portion comprises Amino Acids 1-31 of SEQ ID NO:2.

20. The amino acid sequence of Claim 19 having at least one amino acid substitution.

21. The amino acid sequence of Claim 18, wherein said portion comprises Amino Acids 212-230 of SEQ ID NO:2.

22. The amino acid sequence of Claim 21 having at least one amino acid substitution.

23. The amino acid sequence of Claim 18, wherein said portion comprises an amino acid substitution at Amino Acid 219.

24. The amino acid sequence of Claim 18, wherein said portion consists essentially of Amino Acids 1-446 of SEQ ID NO:2 and wherein said portion behaves as a taxol-resistant beta-tubulin.

25. The amino acid sequence of Claim 24, wherein said portion contains at least one amino acid substitution that alters the taxol binding property of said portion.

26. The amino acid sequence of Claim 24, wherein said portion contains at least one amino acid substitution that does not alter the taxol binding property of said portion.

27. The amino acid sequence of Claim 14, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding Amino Acid 219 as numbered in SEQ ID NO:2.

28. A purified DNA segment encoding a beta-tubulin of the fungal species *Pythium ultimum* or a portion thereof.

29. The DNA segment of Claim 28, wherein said portion encodes at least one taxol binding site.

30. The DNA segment of Claim 29, wherein said portion encodes a protein having taxol binding site I and taxol binding site II.

31. The DNA segment of Claim 30, wherein said protein is able to interact with alpha-tubulin.

32. The DNA segment of Claim 28, wherein said DNA segment comprises at least a portion of SEQ ID NO:3.

33. The DNA segment of Claim 32, wherein said portion comprises the nucleotide sequence from nucleotide 92 through nucleotide 184 of SEQ ID NO:3.

34. The DNA segment of Claim 33, wherein at least one nucleotide in said nucleotide sequence is substituted.

35. The DNA segment of Claim 32, wherein said portion comprises the nucleotide sequence from nucleotide 725 through nucleotide 781 of SEQ ID NO:3

36. The DNA segment of Claim 35, wherein at least one nucleotide in said nucleotide sequence is substituted.

37. The DNA segment of Claim 35, wherein nucleotide 746, nucleotide 747, nucleotide 748 or mixtures thereof are substituted.

38. The DNA segment of Claim 32, comprising the nucleotide sequence from nucleotide 92 to nucleotide 1429 of SEQ ID NO:3, said DNA segment encoding a beta-tubulin.

39. The DNA segment of Claim 38, wherein at least one nucleotide in said nucleotide sequence is substituted and wherein the taxol binding capacity of said beta-tubulin is not altered.

40. The DNA segment of Claim 38, wherein at least one nucleotide in said nucleotide sequence is substituted and wherein the taxol binding capacity of said beta-tubulin is altered.

41. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Pythium ultimum*.

42. The amino acid sequence of Claim 41, wherein said amino acid sequence comprises at least one taxol binding site.

43. The amino acid sequence of Claim 42, wherein said portion comprises taxol binding site I and taxol binding site II.

44. The amino acid sequence of Claim 43, wherein said portion is able to interact with alpha-tubulin.

45. The amino acid sequence of Claim 41, wherein said amino acid sequence comprises at least a portion of the beta-tubulin as depicted in SEQ ID NO:4.

46. The amino acid sequence of Claim 45, wherein said portion comprises Amino Acids 1-31 of SEQ ID NO:4

47. The amino acid sequence of Claim 46, having at least one amino acid substitution.

48. The amino acid sequence of Claim 45, wherein said portion comprises Amino Acids 212-230 of SEQ ID NO:4

49. The amino acid sequence of Claim 48, having at least one amino acid substitution.

50. The amino acid sequence of Claim 45, wherein said portion comprises an amino acid substitution at Amino Acid 219.

51. The amino acid sequence of Claim 45, wherein said portion consists essentially of Amino Acids 1-446 of SEQ ID NO:4 and wherein said portion behaves as a taxol-sensitive beta-tubulin.

52. The amino acid sequence of Claim 51, wherein said portion contains at least one amino acid substitution that alters the taxol binding property of said portion.

53. The amino acid sequence of Claim 51, wherein said portion contains at least one amino acid substitution that does not alter the taxol binding property of said portion.

54. The amino acid sequence of Claim 41, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding Amino Acid 219 as numbered in SEQ ID NO:4.

55. A purified DNA segment encoding a beta-tubulin of the fungal species *Phytophthora cinnamomi* or a portion thereof, wherein said DNA segment consists essentially of at least a portion of SEQ ID NO:5.

56. The DNA segment of Claim 55, wherein said portion comprises the nucleotide sequence from nucleotide 11 through nucleotide 103 of SEQ ID NO:5.

57. The DNA segment of Claim 56, wherein at least one nucleotide in said nucleotide sequence is substituted, providing that when nucleotide substitution changes only

one amino acid code nucleotide 80 cannot consist of adenine while nucleotide 81 is thymine and nucleotide 82 is adenine, cytosine or thymine.

58. The DNA segment of Claim 55, wherein said portion comprises the nucleotide sequence from nucleotide 644 through nucleotide 700 of SEQ ID NO:5

59. The DNA segment of Claim 58, wherein at least one nucleotide in said nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code nucleotide 665 cannot be adenine while nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine.

60. The DNA segment of Claim 55, comprising the nucleotide sequence from nucleotide 11 to nucleotide 1342 of SEQ ID NO:5, said DNA segment encoding a beta-tubulin.

61. The DNA segment of Claim 60, wherein at least one nucleotide in said nucleotide sequence is substituted, providing that when nucleotide substitution changes only one amino acid code nucleotide 665 cannot be adenine while nucleotide 666 is adenine and nucleotide 667 is cytosine or thymine.

62. The DNA segment of Claim 60 or 61, wherein at least one nucleotide in said nucleotide sequence is substituted, and wherein the taxol binding capacity of said beta-tubulin is not altered.

63. The DNA segment of Claim 60 or 61, wherein at least one nucleotide in said nucleotide sequence is substituted and wherein the taxol binding capacity of said beta-tubulin is altered.

64. An amino acid sequence comprising at least a portion of a beta-tubulin of the fungal species *Phytophthora cinnamomi* as depicted in SEQ ID NO:6.

65. The amino acid sequence of Claim 64, wherein said portion comprises Amino Acids 1-31 of SEQ ID NO:6.

66. The amino acid sequence of Claim 65, having at least one amino acid is substituted, providing that when only one amino acid is substituted Amino Acid 24 is not isoleucine.

67. The amino acid sequence of Claim 64, wherein said portion comprises Amino Acids 212-230 of SEQ ID NO:6.

68. The amino acid sequence of Claim 67, having at least one amino acid is substituted, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine.

69. The amino acid sequence of Claim 64, wherein said portion comprises an amino acid substitution at Amino Acid 219, wherein said Amino Acid 219 is not substituted with asparagine.

70. The amino acid sequence of Claim 64, wherein said portion consists essentially of Amino Acids 1-446 of SEQ ID NO:6 and wherein said portion behaves as a taxol-sensitive beta-tubulin.

71. The amino acid sequence of Claim 70, wherein said portion contains at least one amino acid substitution, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine, and wherein said amino acid substitution that alters the taxol binding property of said portion.

72. The amino acid sequence of Claim 70, wherein said portion contains at least one amino acid substitution, providing that when only one amino acid is substituted Amino Acid 219 is not asparagine, and wherein said amino acid substitution does not alter the taxol binding property of said portion.

73. The amino acid sequence of Claim 64, wherein said amino acid sequence is substituted with any amino acid which perturbs the three-dimensional structure of said amino acid sequence surrounding Amino Acid 219 and wherein when only one amino acid is substituted at Amino Acid 219 said substituted amino acid is not asparagine.

74. A vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Pestalotiopsis microspora* or a portion thereof.

75. The vector of Claim 74, wherein said portion encodes at least one taxol binding site.

76. A vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Pythium ultimum* or a portion thereof.

77. The vector of Claim 76, wherein said portion encodes at least one taxol binding site.

78. A vector comprising a purified DNA segment encoding a beta-tubulin of the fungal species *Phytophthora cinnamomi* wherein said DNA segment consists essentially of SEQ ID No:5 or a portion thereof.

79. The vector of Claim 78, wherein said portion encodes at least one taxol binding site.

80. A method of determining the taxol binding capacity of a beta-tubulin or beta-tubulin-like compound comprising

providing antibodies raised against amino acid sequences comprising at least one taxol binding site of a beta-tubulin from a taxol-resistant *Pestalotiopsis microspora,* a taxol-sensitive *Pythium ultimum,* or taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6 to form a reagent, wherein said antibodies distinguish between taxol-binding and non-taxol-binding properties;

contacting said beta-tubulin with said reagent; and

determining degree of binding between said antibodies in said reagent and said beta-tubulin or beta-tubulin-like compound;

whereby binding of antibodies which specifically recognize taxol-binding properties indicate taxol sensitive; whereby binding of antibodies which specifically recognize taxol-non-binding properties indicate taxol resistance.

81. The method of Claim 80, wherein said antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-resistant *Pestalotiopsis microspora.*

82. The method of Claim 81, wherein said amino acid sequence comprises at least one taxol binding site from SEQ ID NO:2

83. The method of Claim 80, wherein said antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-sensitive *Pythium ultimum.*

84. The method of Claim 81, wherein said amino acid sequence comprises at least one taxol binding site from SEQ ID NO:4

85. The method of Claim 80, wherein said antibodies are raised against an amino acid sequence comprising at least one taxol binding site of a beta-tubulin from a taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6.

86. The method of Claims 80, 81, 82, 83, 84 or 85, wherein said beta-tubulin or beta-tubulin-like protein is selected from the group consisting of recombinantly expressed protein, exogenously isolated protein, synthetic peptides, and cell cultures.

87. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

providing beta-tubulins with amino acid sequences comprising both taxol binding sites from taxol-sensitive *Pythium ultimum* or taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6 in addition to alpha-tubulin from any taxol-sensitive organism to form a reagent;

contacting said composition of matter with said reagent; and

determining the ability of the composition of matter to promote MT assembly or ability to prevent depolymerization of assembled MTs under depolymerizing conditions;

whereby the ability to promote microtubule assembly or prevent depolymerization indicate the possible presence of taxol or taxol-like compounds in said composition of matter.

88. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

providing mycelia of taxol-sensitive *Pythium ultimum* or a taxol-sensitive *Phytophthora cinnamomi* as depicted in SEQ ID NO:6;

contacting said composition of matter with said mycelia in the presence of said labeled taxol; and

determining degree of competitive inhibition of binding between said beta-tubulins and said labeled taxol by said composition of matter;

whereby the composition of matter is determined to possess taxol or taxol-like compounds if it is able to block taxol binding to the beta-tubulins from the taxol-sensitive *Pythium ultimum* or *Phytophthora cinnamomi.*

89. A method of altering the taxol binding property of a recombinantly expressed beta-tubulin or a portion thereof comprising

determining the identity of the codon at Amino Acid 219 as numbered in SEQ ID NO:2 in the coding region of the vector; and

if said codon at Amino Acid 219 codes for any amino acid except threonine, substituting nucleotides in said codon to code for threonine at Amino Acid 219 to alter a non-taxol-binding beta-tubulin or portion thereof to a taxol-binding beta-tubulin or portion thereof or if said codon at Amino Acid 219 codes for threonine, substituting nucleotides in said codon to code for any amino acid except threonine at Amino Acid 219 to alter a taxol-binding beta-tubulin or portion thereof to a non-taxol-binding beta-tubulin or portion thereof.

90. A method of developing a taxol-sensitive fungal cell from a taxol-resistant fungal cell comprising

transforming said non-taxol-sensitive fungal cell by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2;

wherein said transformed fungal cell expresses said DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

91. A transgenic taxol-sensitive fungal cell transformed by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2, wherein said transformed fungal cell expresses said DNA

segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

92. A method of developing a taxol-resistant fungal cell from a taxol-sensitive fungal cell comprising

transforming said taxol-sensitive fungal cell by introducing a DNA segment encoding non-taxol-binding beta-tubulin wherein the amino acid at Amino Acid 219 as numbered in SEQ ID NO:2 is not threonine;

wherein said transformed fungal cell over-expresses said DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

93. A transgenic taxol-sensitive fungal cell transformed by introducing a DNA segment encoding taxol-binding beta-tubulin comprising threonine at Amino Acid 219 as numbered in SEQ ID NO:2, wherein said transformed fungal cell over-expresses said DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.

94. A method of screening a composition of matter for the presence of taxol or taxol-like compounds comprising

providing distinguishable taxol-resistant and taxol-sensitive fungal cells;

contacting said composition of matter with said fungal cells;

determining the growth inhibition of said fungal cells;

whereby the composition of matter is determined to possess taxol or taxol-like compounds if it is able to inhibit the growth of taxol-sensitive fungal cells but not able to inhibit the growth of taxol-resistant fungal cells.

95. The method of Claim 94, wherein said distinguishable taxol-resistant and taxol-sensitive fungal cells consists essentially of transgenic taxol-resistant and taxol-sensitive isogenic fungal cells.

96. The method of Claim 94, wherein said taxol-resistant fungal cells are derived from a fungi which is unrelated to the fungi from which the taxol-sensitive fungal cells are derived.

97. A method for controlling the growth of plant pathogens comprising

determining the taxol sensitivity of said plant pathogen; and

if said pathogen is determined to be taxol-sensitive, said plant and soil surrounding said plant are treated with a taxol-producing *P. microspora.*

98. The method of Claim 97, wherein the taxol sensitivity of said plant pathogen is determined by identifying Amino Acid 219, wherein the plant is designated as taxol-sensitive if Amino Acid 219 is threonine.

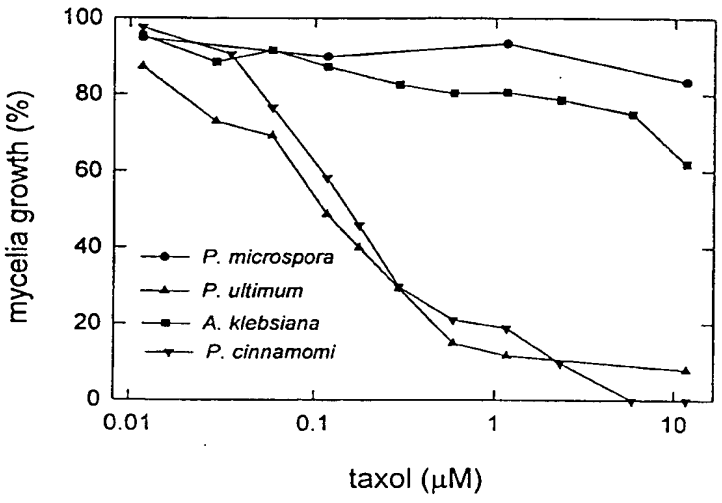
100
80
60
40
20
0
mycelia growth (%)
P. microspora
P. ultimum
A. klebsiana
P. cinnamomi
0.01
0.1
1
10
taxol (μM)

Fig. 1

```
1     CCGTCGAGCTCTACTCCAAAGAAGGCCTCTTTGTTGCTTCCTCTCAGCCTCGACATCTTCTACAAACCGCCATCATGCGTGAGATTGTT
                                                                          M  R  E  I  V    5
90    CACCTCCAGACCGGTCAATGCGGTAACCAAATTGGTGCTGCCTTCTGGCAAACCATCTCTGGCGAGCACGGTCTCGACAGCAATGGAGTC
       H  L  Q  T  G  Q  C  G  N  Q  I  G  A  A  F  W  Q  T  I  S  G  E  H  G  L  D  S  N  G  V   35
180   TACAACGGTACCTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAGGCTTCCGGCAACAAGTACGTTCCTCGTGCCGTCCTC
       Y  N  G  T  S  E  L  Q  L  E  R  M  S  V  Y  F  N  E  A  S  G  N  K  Y  V  P  R  A  V  L   65
270   GTCGATCTCGAGCCCGGTACCATGGATGCCGTCCGCGCCGGTCCTTTCGGTCAGCTCTTCCGCCCTGACAACTTCGTCTTCGGTCAGTCC
       V  D  L  E  P  G  T  M  D  A  V  R  A  G  P  F  G  Q  L  F  R  P  D  N  F  V  F  G  Q  S   95
360   GGTGCCGGAAACAACTGGGCCAAGGGTCACTACACTGAGGGTGCCGAGCTCGTCGACCAGGTCCTCGACGTTGTCCGTCGCGAGGCCGAG
       G  A  G  N  N  W  A  K  G  H  Y  T  E  G  A  E  L  V  D  Q  V  L  D  V  V  R  R  E  A  E  125
450   GCTTGCGACTGCCTCCAGGGTTTCCAGATCACCCACTCCCTGGGTGGTGGTACCGGTGCCGGTATGGGTACTCTGTTGATCTCCAAGATC
       A  C  D  C  L  Q  G  F  Q  I  T  H  S  L  G  G  G  T  G  A  G  M  G  T  L  L  I  S  K  I  155
540   CGTGAGGAGTTCCCCGACCGCATGATGGCCACCTTCTCCGTCGTGCCCTCCCCCAAGGTCTCCGACACCGTCGTCGAGCCCTACAACGCC
       R  E  E  F  P  D  R  M  M  A  T  F  S  V  V  P  S  P  K  V  S  D  T  V  V  E  P  Y  N  A  185
630   ACCCTCTCCGTCCACCAGCTGGTCGAGAACTCGGACGAGACCTTCTGCATTGACAACGAGGCTCTCTACGACATCTGCATGCGCACCCTG
       T  L  S  V  H  Q  L  V  E  N  S  D  E  T  F  C  I  D  N  E  A  L  Y  D  I  C  M  R  T  L  215
720   AAGCTGTCCAACCCCTCGTACGGTGACCTGAACCACCTGGTCTCCGCCGTCATGTCGGGTGTCACCACTTGCTTGCGTTTCCCTGGTCAG
                                                                  NETUB5
       K  L  S  N  P  S  Y  G  D  L  N  H  L  V  S  A  V  M  S  G  V  T  T  C  L  R  F  P  G  Q  245
810   CTGAACTCTGACCTGCGCAAGCTGGCCGTCAACATGGTGCCCTTCCCTCGTCTGCACTTCTTCATGGTCGGCTTTGCTCCCCTGACCAGC
       L  N  S  D  L  R  K  L  A  V  N  M  V  P  F  P  R  L  H  F  F  M  V  G  F  A  P  L  T  S  275
900   CGTGGCGCCCACTCTTTCCGTGCCGTCACCGTCCCCGAGTTGACCCAGCAGATGTTCGACCCCAAGAACATGATGGCTGCTTCCGACTTC
       R  G  A  H  S  F  R  A  V  T  V  P  E  L  T  Q  Q  M  F  D  P  K  N  M  M  A  A  S  D  F  305
990   CGTAACGGTCGCTACCTGACCTGCTCTGCCATCTTCCGTGGTAAGGTCTCCATGAAGGAGGTCGAGGACCAGATGCGCAACGTCCAGAAC
       R  N  G  R  Y  L  T  C  S  A  I  F  R  G  K  V  S  M  K  E  V  E  D  Q  M  R  N  V  Q  N  335
1080  AAGAACTCGTCCTACTTCGTCGAGTGGATCCCCAACAACGTGCAGACCGCCCTCTGCTCCATTCCTCCCCGCGGCCTTAAGATGTCGTCC
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            NETUB6
       T  F  V  G  N  S  T  A  I  Q  E  L  F  K  R  I  G  E  Q  F  T  A  M  F  R  R  K  A  F  L  395
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       H  W  Y  T  G  E  G  M  D  E  M  E  F  T  E  A  E  S  N  M  N  D  L  V  S  E  Y  Q  Q  Y  425
1350  CAGGACGCTGGTGTCGATGAGGAGGAGGAGGAGTACGAGGAGGAGCCTCTGCCCGAGGACGAGTAAACGGCTCGCTAGAGGCTACCAAAG
       Q  D  A  G  V  D  E  E  E  E  E  Y  E  E  E  P  L  P  E  D  E  *                        446
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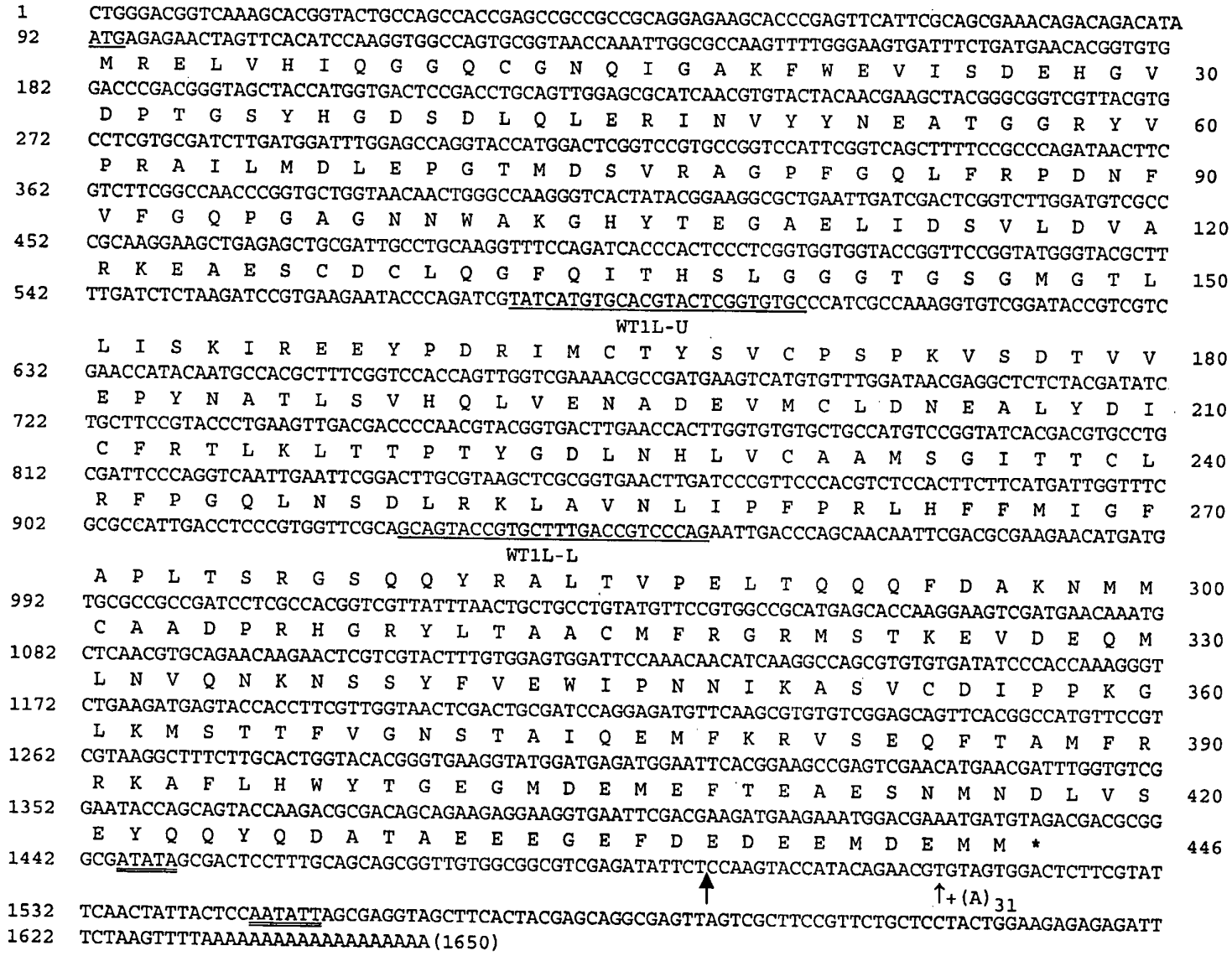
Fig. 2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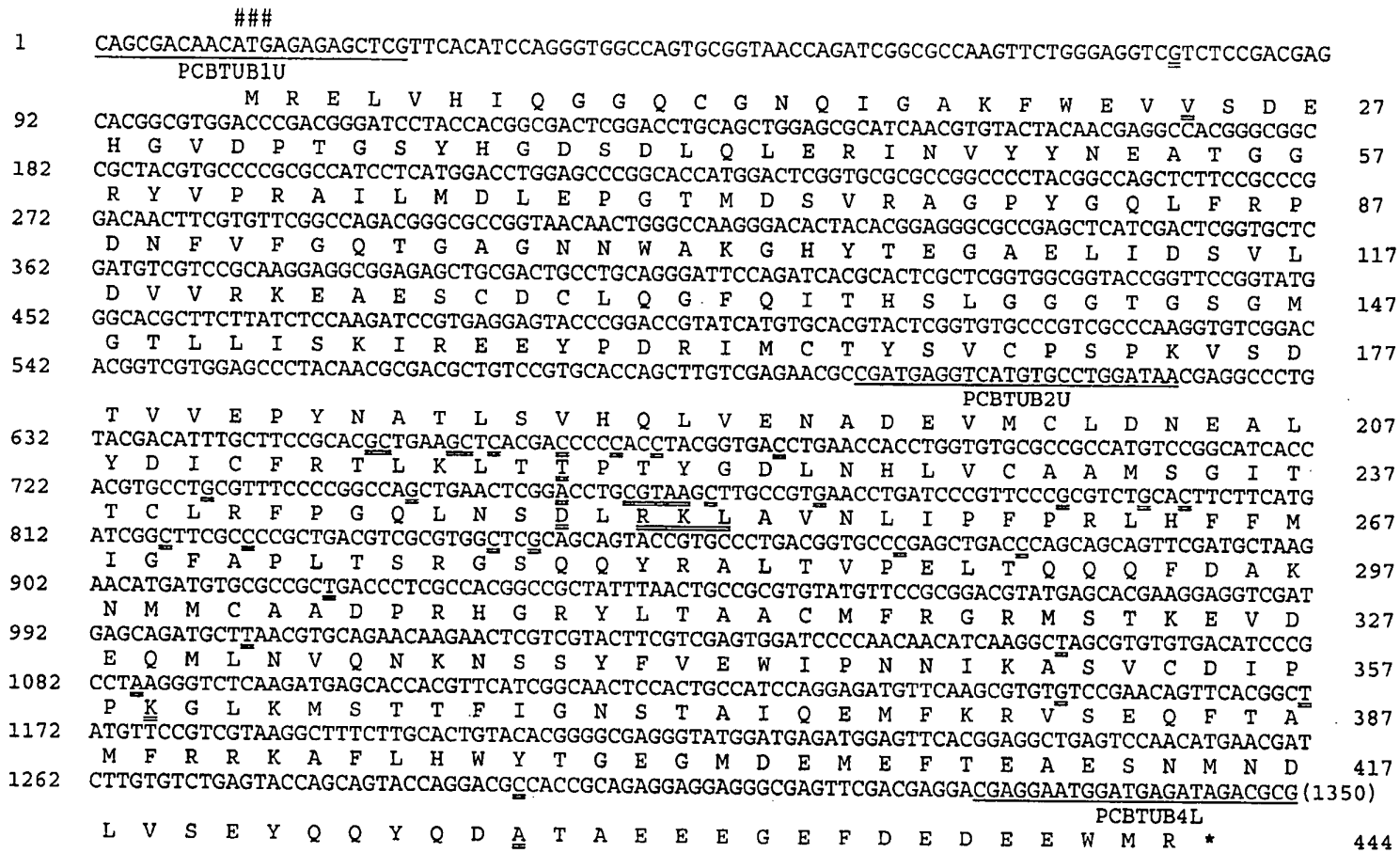
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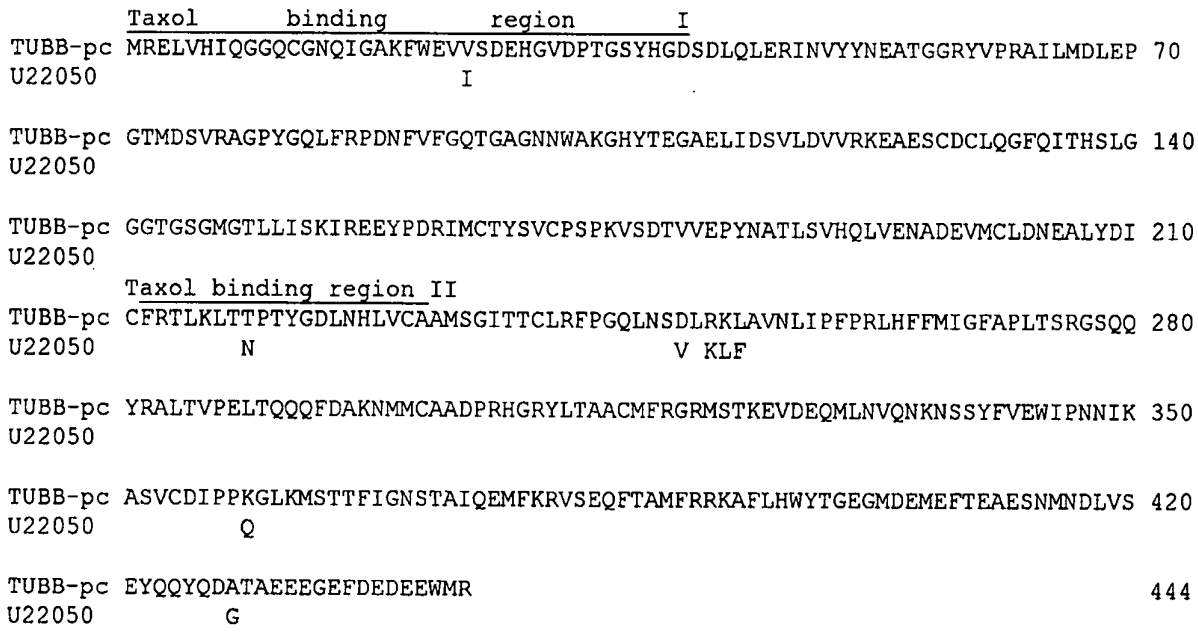
Fig. 4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U22050
TUBB-pc GGTGSGMGTLLISKIREEYPDRIMCTYSVCPSPKVSDTVVEPYNATLSVHQLVENADEVMCLDNEALYDI 210
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TUBB-pc CFRTLKLTTPTYGDLNHLVCAAMSGITTCLRFPGQLNSDLRKLAVNLIPFPRLHFFMIGFAPLTSRGSQQ 280
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U22050 G

Fig. 5

```
                Taxol    binding      region     I
                         *
P. microspora   1 MREIVHLQTGQCGNQIGAAFWQTISGEHGLDSNGVYNGTSELQLERMSVYFNEASGNKYV  60
N. crassa       1                                AS                N           60
A. nidulans     1                                GS            D   N           60
P. ultimum      1   L I G        K EV D  V PT S H D D    IN  Y   T GR  60
P. cinnamomi    1   L I G        K EVV D V PT S H D D    IN  Y   T GR  60
A. klebsiana    1   L I G        K EV D  V PT S H D D    IN  Y   T - T  59
Human β2        1       A        K EV D  I PT T H D D    IN  Y   T G    60

P. microspora   PRAVLVDLEPGTMDAVRAGPFGQLFRPDNFVFGQSGAGNNWAKGHYTEGAELVDQVLDVVRREAE 125
N. crassa                                                               125
A. nidulans                C       E                      N V          125
P. ultimum      I M        S               P               I S   A K    125
P. cinnamomi    I M        S   Y           T               I S L   K    125
A. klebsiana    I M        S   Y           T               I S     K    124
Human β2                   S S     I                       S       K    125
                                           * *
P. microspora   ACDCLQGFQITHSLGGGTGAGMGTLLISKIREEFPDRMMATFSVVPSPKVSDTVVEPYNATLSVH 190
N. crassa       G                                                       190
A. nidulans     G                                                       190
P. ultimum      S                S       Y   I C Y C                    190
P. cinnamomi    S                S       Y   I C Y C                    190
A. klebsiana    S                S       Y   I C Y C                    189
Human β2        S      L         S       Y   I N                        190
                       * *          Taxol binding region  II      *
P. microspora   QLVENSDETFCIDNEALYDICMRTLKLSNPSYGDLNHLVSAVMSGVTTCLRFPGQLNSDLRKLAV 255
N. crassa                                                  VS           255
A. nidulans     H                                                   W   255
P. ultimum      A  VM L          F     TT T          C A   I            255
P. cinnamomi    A  VM L          F     TT T          C A   I            255
A. klebsiana    A  VM L          F     T  T          C A   I  L    V -   253
Human β2        T  Y             F     TT T             T             A  255
```

Fig. 6A

```
                                  # #
P. microspora NMVPFPRLHFFMVGFAPLTSRGAHSFRAVTVPELTQQMFDPKNMMAASDFRNGRYLTCSAIFRGK 320
N. crassa                           H     S                                     320
A. nidulans                         Y     S                                     320
P. ultimum          LI        I     SQQY  L        Q  A    C A P H    AACM    R 320
P. cinnamomi        LI        I     SQQY  L        Q  A    C A P H    AACM    R 320
A. klebsiana        LI        I     SQQY  L        Q  A    C A P H    AACM    R 318
Human β2                      P     SQQY  L           A      C P H    VA V    R 320

                                                    #
P. microspora VSMKEVEDQMRNVQNKNSSYFVEWIPNNVQTALCSIPPRGLKMSSTFVGNSTAIQELFKRIGEQF 385
N. crassa                                                                     385
A. nidulans               I S  Q              I S          I    S       V D    385
P. ultimum          M T   DE  L              IKASV D   K    T              M   VS    385
P. cinnamomi        M T   DE  L              IKASV D   K    T  I           M   VS    385
A. klebsiana        M T   DE  L              IKASV D   K    T  I           M   VS    383
Human β2            M     DE  L                 K  V D        A  I              S    385


P. microspora TAMFRRKAFLHWYTGEGMDEMEFTEAESNMNDLVSEYQQYQDAGVDEEEEEYEEEP-LPEDE$  446
N. crassa                                                          A-PL G E$  447
A. nidulans                                 SIS G     A   E-IM G E$           447
P. ultimum                                  TAE -   G FD DEEMD MM$           446
P. cinnamomi                                TAE -   G FD DEEWMR$             444
A. klebsiana                                TAE -   G FD DEEMD MM$           444
Human β2                                    TAE -   G F   AEEEVA$             445
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Fig. 6B

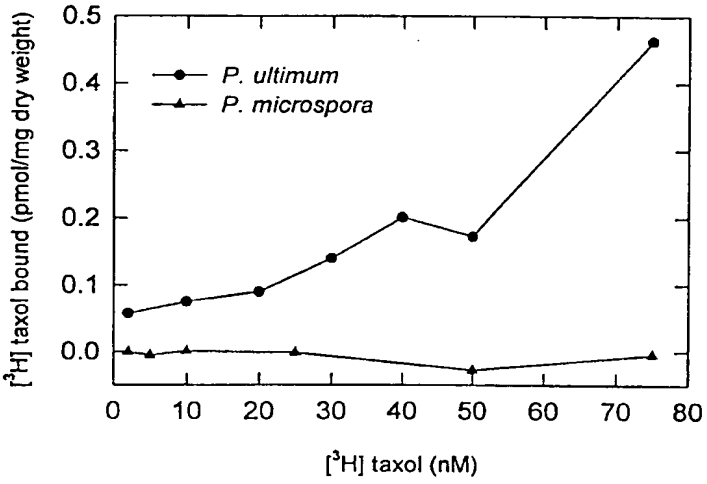
0.5
0.4
0.3
0.2
0.1
0.0
P. ultimum
P. microspora
[3H] taxol bound (pmol/mg dry weight)
0
10
20
30
40
50
60
70
80
[3H] taxol (nM)

Fig. 7A

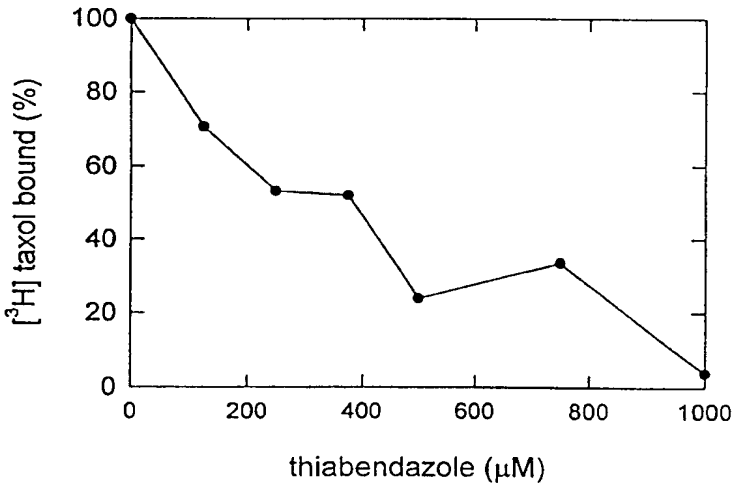
100
80
60
40
20
0
[3H] taxol bound (%)
0
200
400
600
800
1000
thiabendazole (μM)

Fig.7B

	Region I (1-31 aa)	Region II (212-231 aa)
	**********	******
(s) Pig	MREIVHIQAGQCGNQIGAKFWEVISDEHGID	FRTLKLTTPTYGDLNHLVSA
(s) Human β2	L	
(s) *Drosophila* β1	I	L
(s) *Xenopus* β4	L	
(s) *Tetrahymena*	G	
(s) *Physarum* β1		
(s) *P. ultimum*	L G V	C
(s) *P. cinnamomi*	L G V V	C
(r) *A. klebsiana*	L G V N	C
(r) *P. microspora*	L T A QT G L M SN S	
(r) *A. nidulans* benA	L T A QT G L M SN S	
(r) *S. cerevisiae*	I SA Y A T CG L Q NQ S N S	

Fig.8

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Bollon, Arthur P.
Mu, Jing-Hong
Cytoclonal Pharmaceutics, Inc.

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30 35 40

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Gly Ala Glu Leu Val Asp Gln Val Leu Asp Val Val Arg Arg Glu Ala
     110                 115                 120

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Glu Ala Cys Asp Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly
125                 130                 135                 140

ggt ggt acc ggt gcc ggt atg ggt act ctg ttg atc tcc aag atc cgt   542
Gly Gly Thr Gly Ala Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg
                145                 150                 155

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Pro Lys Val Ser Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser
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Val His Gln Leu Val Glu Asn Ser Asp Glu Thr Phe Cys Ile Asp Asn
    190                 195                 200

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Glu Ala Leu Tyr Asp Ile Cys Met Arg Thr Leu Lys Leu Ser Asn Pro
205                 210                 215                 220

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Ser Tyr Gly Asp Leu Asn His Leu Val Ser Ala Val Met Ser Gly Val
                225                 230                 235

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Thr Thr Cys Leu Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys
            240                 245                 250

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Thr Val Pro Glu Leu Thr Gln Gln Met Phe Asp Pro Lys Asn Met Met
285                 290                 295                 300

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                305                 310                 315
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Val Gln Asn Lys Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn
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365                 370                 375                 380

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                385                 390                 395

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Trp Tyr Thr Gly Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu
            400                 405                 410

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Ser Asn Met Asn Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala
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Asp Glu
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Met Arg Glu Ile Val His Leu Gln Thr Gly Gln Cys Gly Asn Gln Ile
  1               5                  10                  15
```

```
Gly Ala Ala Phe Trp Gln Thr Ile Ser Gly Glu His Gly Leu Asp Ser
            20                  25                  30

Asn Gly Val Tyr Asn Gly Thr Ser Glu Leu Gln Leu Glu Arg Met Ser
        35                  40                  45

Val Tyr Phe Asn Glu Ala Ser Gly Asn Lys Tyr Val Pro Arg Ala Val
    50                  55                  60

Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ala Val Arg Ala Gly Pro
65                  70                  75                  80

Phe Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser Gly
                85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Val Asp Gln Val Leu Asp Val Val Arg Arg Glu Ala Glu Ala Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ala Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Phe Pro
145                 150                 155                 160

Asp Arg Met Met Ala Thr Phe Ser Val Val Pro Ser Pro Lys Val Ser
                165                 170                 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
            180                 185                 190

Val Glu Asn Ser Asp Glu Thr Phe Cys Ile Asp Asn Glu Ala Leu Tyr
        195                 200                 205

Asp Ile Cys Met Arg Thr Leu Lys Leu Ser Asn Pro Ser Tyr Gly Asp
    210                 215                 220

Leu Asn His Leu Val Ser Ala Val Met Ser Gly Val Thr Thr Cys Leu
225                 230                 235                 240

Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu Ala Val Asn
                245                 250                 255

Met Val Pro Phe Pro Arg Leu His Phe Phe Met Val Gly Phe Ala Pro
            260                 265                 270

Leu Thr Ser Arg Gly Ala His Ser Phe Arg Ala Val Thr Val Pro Glu
        275                 280                 285

Leu Thr Gln Gln Met Phe Asp Pro Lys Asn Met Met Ala Ala Ser Asp
    290                 295                 300
```

```
Phe Arg Asn Gly Arg Tyr Leu Thr Cys Ser Ala Ile Phe Arg Gly Lys
305                 310                 315                 320

Val Ser Met Lys Glu Val Glu Asp Gln Met Arg Asn Val Gln Asn Lys
                325                 330                 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Val Gln Thr Ala
            340                 345                 350

Leu Cys Ser Ile Pro Pro Arg Gly Leu Lys Met Ser Ser Thr Phe Val
        355                 360                 365

Gly Asn Ser Thr Ala Ile Gln Glu Leu Phe Lys Arg Ile Gly Glu Gln
    370                 375                 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385                 390                 395                 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
                405                 410                 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Gly Val Asp Glu
            420                 425                 430

Glu Glu Glu Glu Tyr Glu Glu Glu Pro Leu Pro Glu Asp Glu
        435                 440                 445
```

<210> 3
<211> 1650
<212> DNA
<213> Pythium ultimum

<220>
<221> CDS
<222> (92)..(1429)

<400> 3

```
ctgggacggt caaagcacgg tactgccagc caccgagccg ccgccgcagg agaagcaccc 60

gagttcattc gcagcgaaac agacagacat a atg aga gaa cta gtt cac atc    112
                                    Met Arg Glu Leu Val His Ile
                                      1               5

caa ggt ggc cag tgc ggt aac caa att ggc gcc aag ttt tgg gaa gtg   160
Gln Gly Gly Gln Cys Gly Asn Gln Ile Gly Ala Lys Phe Trp Glu Val
         10                  15                  20

att tct gat gaa cac ggt gtg gac ccg acg ggt agc tac cat ggt gac   208
Ile Ser Asp Glu His Gly Val Asp Pro Thr Gly Ser Tyr His Gly Asp
     25                  30                  35
```

```
tcc gac ctg cag ttg gag cgc atc aac gtg tac tac aac gaa gct acg   256
Ser Asp Leu Gln Leu Glu Arg Ile Asn Val Tyr Tyr Asn Glu Ala Thr
 40                  45                  50                  55

ggc ggt cgt tac gtg cct cgt gcg atc ttg atg gat ttg gag cca ggt   304
Gly Gly Arg Tyr Val Pro Arg Ala Ile Leu Met Asp Leu Glu Pro Gly
                 60                  65                  70

acc atg gac tcg gtc cgt gcc ggt cca ttc ggt cag ctt ttc cgc cca   352
Thr Met Asp Ser Val Arg Ala Gly Pro Phe Gly Gln Leu Phe Arg Pro
             75                  80                  85

gat aac ttc gtc ttc ggc caa ccc ggt gct ggt aac aac tgg gcc aag   400
Asp Asn Phe Val Phe Gly Gln Pro Gly Ala Gly Asn Asn Trp Ala Lys
         90                  95                 100

ggt cac tat acg gaa ggc gct gaa ttg atc gac tcg gtc ttg gat gtc   448
Gly His Tyr Thr Glu Gly Ala Glu Leu Ile Asp Ser Val Leu Asp Val
    105                 110                 115

gcc cgc aag gaa gct gag agc tgc gat tgc ctg caa ggt ttc cag atc   496
Ala Arg Lys Glu Ala Glu Ser Cys Asp Cys Leu Gln Gly Phe Gln Ile
120                 125                 130                 135

acc cac tcc ctc ggt ggt ggt acc ggt tcc ggt atg ggt acg ctt ttg   544
Thr His Ser Leu Gly Gly Gly Thr Gly Ser Gly Met Gly Thr Leu Leu
                140                 145                 150

atc tct aag atc cgt gaa gaa tac cca gat cgt atc atg tgc acg tac   592
Ile Ser Lys Ile Arg Glu Glu Tyr Pro Asp Arg Ile Met Cys Thr Tyr
            155                 160                 165

tcg gtg tgc cca tcg cca aag gtg tcg gat acc gtc gtc gaa cca tac   640
Ser Val Cys Pro Ser Pro Lys Val Ser Asp Thr Val Val Glu Pro Tyr
        170                 175                 180

aat gcc acg ctt tcg gtc cac cag ttg gtc gaa aac gcc gat gaa gtc   688
Asn Ala Thr Leu Ser Val His Gln Leu Val Glu Asn Ala Asp Glu Val
    185                 190                 195

atg tgt ttg gat aac gag gct ctc tac gat atc tgc ttc cgt acc ctg   736
Met Cys Leu Asp Asn Glu Ala Leu Tyr Asp Ile Cys Phe Arg Thr Leu
200                 205                 210                 215

aag ttg acg acc cca acg tac ggt gac ttg aac cac ttg gtg tgt gct   784
Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His Leu Val Cys Ala
                220                 225                 230

gcc atg tcc ggt atc acg acg tgc ctg cga ttc cca ggt caa ttg aat   832
Ala Met Ser Gly Ile Thr Thr Cys Leu Arg Phe Pro Gly Gln Leu Asn
            235                 240                 245

tcg gac ttg cgt aag ctc gcg gtg aac ttg atc ccg ttc cca cgt ctc   880
Ser Asp Leu Arg Lys Leu Ala Val Asn Leu Ile Pro Phe Pro Arg Leu
        250                 255                 260
```

```
cac ttc ttc atg att ggt ttc gcg cca ttg acc tcc cgt ggt tcg cag   928
His Phe Phe Met Ile Gly Phe Ala Pro Leu Thr Ser Arg Gly Ser Gln
    265                 270                 275

cag tac cgt gct ttg acc gtc cca gaa ttg acc cag caa caa ttc gac   976
Gln Tyr Arg Ala Leu Thr Val Pro Glu Leu Thr Gln Gln Gln Phe Asp
280                 285                 290                 295

gcg aag aac atg atg tgc gcc gcc gat cct cgc cac ggt cgt tat tta   1024
Ala Lys Asn Met Met Cys Ala Ala Asp Pro Arg His Gly Arg Tyr Leu
                300                 305                 310

act gct gcc tgt atg ttc cgt ggc cgc atg agc acc aag gaa gtc gat   1072
Thr Ala Ala Cys Met Phe Arg Gly Arg Met Ser Thr Lys Glu Val Asp
            315                 320                 325

gaa caa atg ctc aac gtg cag aac aag aac tcg tcg tac ttt gtg gag   1120
Glu Gln Met Leu Asn Val Gln Asn Lys Asn Ser Ser Tyr Phe Val Glu
        330                 335                 340

tgg att cca aac aac atc aag gcc agc gtg tgt gat atc cca cca aag   1168
Trp Ile Pro Asn Asn Ile Lys Ala Ser Val Cys Asp Ile Pro Pro Lys
    345                 350                 355

ggt ctg aag atg agt acc acc ttc gtt ggt aac tcg act gcg atc cag   1216
Gly Leu Lys Met Ser Thr Thr Phe Val Gly Asn Ser Thr Ala Ile Gln
360                 365                 370                 375

gag atg ttc aag cgt gtg tcg gag cag ttc acg gcc atg ttc cgt cgt   1264
Glu Met Phe Lys Arg Val Ser Glu Gln Phe Thr Ala Met Phe Arg Arg
                380                 385                 390

aag gct ttc ttg cac tgg tac acg ggt gaa ggt atg gat gag atg gaa   1312
Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Gly Met Asp Glu Met Glu
            395                 400                 405

ttc acg gaa gcc gag tcg aac atg aac gat ttg gtg tcg gaa tac cag   1360
Phe Thr Glu Ala Glu Ser Asn Met Asn Asp Leu Val Ser Glu Tyr Gln
        410                 415                 420

cag tac caa gac gcg aca gca gaa gag gaa ggt gaa ttc gac gaa gat   1408
Gln Tyr Gln Asp Ala Thr Ala Glu Glu Glu Gly Glu Phe Asp Glu Asp
    425                 430                 435

gaa gaa atg gac gaa atg atg tagacgacgc gggcgatata gcgactcctt      1459
Glu Glu Met Asp Glu Met Met
440                 445

tgcagcagcg gttgtggcgg cgtcgagata ttctccaagt accatacaga acgtgtagtg 1519

gactcttcgt attcaactat tactccaata ttagcgaggt agcttcacta cgagcaggcg 1579

agttagtcgc ttccgttctg ctcctactgg aagagagaga tttctaagtt ttaaaaaaaa 1639

aaaaaaaaaa a                                                      1650
```

<210> 4
<211> 446
<212> PRT
<213> Pythium ultimum

<400> 4

```
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
  1               5                  10                  15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Val Asp Pro
            20                  25                  30

Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile Asn
        35                  40                  45

Val Tyr Tyr Asn Glu Ala Thr Gly Gly Arg Tyr Val Pro Arg Ala Ile
    50                  55                  60

Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ala Gly Pro
65                  70                  75                  80

Phe Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Pro Gly
                85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Ile Asp Ser Val Leu Asp Val Ala Arg Lys Glu Ala Glu Ser Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro
145                 150                 155                 160

Asp Arg Ile Met Cys Thr Tyr Ser Val Cys Pro Ser Pro Lys Val Ser
                165                 170                 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
            180                 185                 190

Val Glu Asn Ala Asp Glu Val Met Cys Leu Asp Asn Glu Ala Leu Tyr
        195                 200                 205

Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp
    210                 215                 220

Leu Asn His Leu Val Cys Ala Ala Met Ser Gly Ile Thr Thr Cys Leu
225                 230                 235                 240

Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu Ala Val Asn
                245                 250                 255
```

```
Leu Ile Pro Phe Pro Arg Leu His Phe Phe Met Ile Gly Phe Ala Pro
            260                 265                 270

Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro Glu
        275                 280                 285

Leu Thr Gln Gln Gln Phe Asp Ala Lys Asn Met Met Cys Ala Ala Asp
    290                 295                 300

Pro Arg His Gly Arg Tyr Leu Thr Ala Ala Cys Met Phe Arg Gly Arg
305                 310                 315                 320

Met Ser Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys
                325                 330                 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile Lys Ala Ser
            340                 345                 350

Val Cys Asp Ile Pro Pro Lys Gly Leu Lys Met Ser Thr Thr Phe Val
        355                 360                 365

Gly Asn Ser Thr Ala Ile Gln Glu Met Phe Lys Arg Val Ser Glu Gln
    370                 375                 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385                 390                 395                 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
                405                 410                 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala Glu Glu
            420                 425                 430

Glu Gly Glu Phe Asp Glu Asp Glu Glu Met Asp Glu Met Met
        435                 440                 445
```

<210> 5
<211> 1350
<212> DNA
<213> Phytophthora cinnamomi

<220>
<221> CDS
<222> (11)..(1342)

<400> 5

```
cagcgacaac atg aga gag ctc gtt cac atc cag ggt ggc cag tgc ggt    49
           Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly
             1               5                  10

aac cag atc ggc gcc aag ttc tgg gag gtc gtc tcc gac gag cac ggc   97
Asn Gln Ile Gly Ala Lys Phe Trp Glu Val Val Ser Asp Glu His Gly
     15                  20                  25
```

```
gtg gac ccg acg gga tcc tac cac ggc gac tcg gac ctg cag ctg gag   145
Val Asp Pro Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu
 30                  35                  40                  45

cgc atc aac gtg tac tac aac gag gcc acg ggc ggc cgc tac gtg ccc   193
Arg Ile Asn Val Tyr Tyr Asn Glu Ala Thr Gly Gly Arg Tyr Val Pro
                 50                  55                  60

cgc gcc atc ctc atg gac ctg gag ccc ggc acc atg gac tcg gtg cgc   241
Arg Ala Ile Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg
             65                  70                  75

gcc ggc ccc tac ggc cag ctc ttc cgc ccg gac aac ttc gtg ttc ggc   289
Ala Gly Pro Tyr Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly
         80                  85                  90

cag acg ggc gcc ggt aac aac tgg gcc aag gga cac tac acg gag ggc   337
Gln Thr Gly Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly
     95                 100                 105

gcc gag ctc atc gac tcg gtg ctc gat gtc gtc cgc aag gag gcg gag   385
Ala Glu Leu Ile Asp Ser Val Leu Asp Val Val Arg Lys Glu Ala Glu
110                 115                 120                 125

agc tgc gac tgc ctg cag gga ttc cag atc acg cac tcg ctc ggt ggc   433
Ser Cys Asp Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly
                130                 135                 140

ggt acc ggt tcc ggt atg ggc acg ctt ctt atc tcc aag atc cgt gag   481
Gly Thr Gly Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu
            145                 150                 155

gag tac ccg gac cgt atc atg tgc acg tac tcg gtg tgc ccg tcg ccc   529
Glu Tyr Pro Asp Arg Ile Met Cys Thr Tyr Ser Val Cys Pro Ser Pro
        160                 165                 170

aag gtg tcg gac acg gtc gtg gag ccc tac aac gcg acg ctg tcc gtg   577
Lys Val Ser Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val
    175                 180                 185

cac cag ctt gtc gag aac gcc gat gag gtc atg tgc ctg gat aac gag   625
His Gln Leu Val Glu Asn Ala Asp Glu Val Met Cys Leu Asp Asn Glu
190                 195                 200                 205

gcc ctg tac gac att tgc ttc cgc acg ctg aag ctc acg acc ccc acc   673
Ala Leu Tyr Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr
                210                 215                 220

tac ggt gac ctg aac cac ctg gtg tgc gcc gcc atg tcc ggc atc acc   721
Tyr Gly Asp Leu Asn His Leu Val Cys Ala Ala Met Ser Gly Ile Thr
            225                 230                 235

acg tgc ctg cgt ttc ccc ggc cag ctg aac tcg gac ctg cgt aag ctt   769
Thr Cys Leu Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu
        240                 245                 250
```

```
gcc gtg aac ctg atc ccg ttc ccg cgt ctg cac ttc ttc atg atc ggc   817
Ala Val Asn Leu Ile Pro Phe Pro Arg Leu His Phe Phe Met Ile Gly
    255                 260                 265

ttc gcc ccg ctg acg tcg cgt ggc tcg cag cag tac cgt gcc ctg acg   865
Phe Ala Pro Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr
270                 275                 280                 285

gtg ccc gag ctg acc cag cag cag ttc gat gct aag aac atg atg tgc   913
Val Pro Glu Leu Thr Gln Gln Gln Phe Asp Ala Lys Asn Met Met Cys
                290                 295                 300

gcc gct gac cct cgc cac ggc cgc tat tta act gcc gcg tgt atg ttc   961
Ala Ala Asp Pro Arg His Gly Arg Tyr Leu Thr Ala Ala Cys Met Phe
            305                 310                 315

cgc gga cgt atg agc acg aag gag gtc gat gag cag atg ctt aac gtg   1009
Arg Gly Arg Met Ser Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val
        320                 325                 330

cag aac aag aac tcg tcg tac ttc gtc gag tgg atc ccc aac aac atc   1057
Gln Asn Lys Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile
    335                 340                 345

aag gct agc gtg tgt gac atc ccg cct aag ggt ctc aag atg agc acc   1105
Lys Ala Ser Val Cys Asp Ile Pro Pro Lys Gly Leu Lys Met Ser Thr
350                 355                 360                 365

acg ttc atc ggc aac tcc act gcc atc cag gag atg ttc aag cgt gtg   1153
Thr Phe Ile Gly Asn Ser Thr Ala Ile Gln Glu Met Phe Lys Arg Val
                370                 375                 380

tcc gaa cag ttc acg gct atg ttc cgt cgt aag gct ttc ttg cac tgt   1201
Ser Glu Gln Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Cys
            385                 390                 395

aca cgg ggc gag ggt atg gat gag atg gag ttc acg gag gct gag tcc   1249
Thr Arg Gly Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser
        400                 405                 410

aac atg aac gat ctt gtg tct gag tac cag cag tac cag gac gcc acc   1297
Asn Met Asn Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr
    415                 420                 425

gca gag gag gag ggc gag ttc gac gag gac gag gaa tgg atg aga       1342
Ala Glu Glu Glu Gly Glu Phe Asp Glu Asp Glu Glu Trp Met Arg
430                 435                 440

tagacgcg                                                         1350
```

<210> 6
<211> 444
<212> PRT
<213> Phytophthora cinnamomi

<400> 6

```
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
  1               5                  10                  15

Gly Ala Lys Phe Trp Glu Val Val Ser Asp Glu His Gly Val Asp Pro
             20                  25                  30

Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile Asn
         35                  40                  45

Val Tyr Tyr Asn Glu Ala Thr Gly Gly Arg Tyr Val Pro Arg Ala Ile
     50                  55                  60

Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ala Gly Pro
 65                  70                  75                  80

Tyr Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Thr Gly
                 85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Ile Asp Ser Val Leu Asp Val Val Arg Lys Glu Ala Glu Ser Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro
145                 150                 155                 160

Asp Arg Ile Met Cys Thr Tyr Ser Val Cys Pro Ser Pro Lys Val Ser
                165                 170                 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
            180                 185                 190

Val Glu Asn Ala Asp Glu Val Met Cys Leu Asp Asn Glu Ala Leu Tyr
        195                 200                 205

Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp
    210                 215                 220

Leu Asn His Leu Val Cys Ala Ala Met Ser Gly Ile Thr Thr Cys Leu
225                 230                 235                 240

Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu Ala Val Asn
                245                 250                 255
```

```
Leu Ile Pro Phe Pro Arg Leu His Phe Phe Met Ile Gly Phe Ala Pro
            260                 265                 270

Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro Glu
        275                 280                 285

Leu Thr Gln Gln Gln Phe Asp Ala Lys Asn Met Met Cys Ala Ala Asp
    290                 295                 300

Pro Arg His Gly Arg Tyr Leu Thr Ala Ala Cys Met Phe Arg Gly Arg
305                 310                 315                 320

Met Ser Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys
                325                 330                 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile Lys Ala Ser
            340                 345                 350

Val Cys Asp Ile Pro Pro Lys Gly Leu Lys Met Ser Thr Thr Phe Ile
        355                 360                 365

Gly Asn Ser Thr Ala Ile Gln Glu Met Phe Lys Arg Val Ser Glu Gln
    370                 375                 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Cys Thr Arg Gly
385                 390                 395                 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
                405                 410                 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala Glu Glu
            420                 425                 430

Glu Gly Glu Phe Asp Glu Asp Glu Glu Trp Met Arg
        435                 440
```

<210> 7
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward degenerate primer

<400> 7
ctgggcyaag ggycaytaca cygag 25

<210> 8
<211> 8
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum

<400> 8
Trp Ala Lys Gly His Tyr Thr Glu
1 5

<210> 9
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (14)
<223> i

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 9
cgaagaartg rarncgrggg aargg 25

<210> 10
<211> 8
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum

<400> 10
Pro Phe Pro Arg Leu His Phe Phe
1 5

<210> 11
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

<400> 11
cgagccytac aacgcyacyc t 21

<210> 12
<211> 7
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum

<400> 12
Glu Pro Tyr Asn Ala Thr Leu
1 5

<210> 13
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 13
ctcgttcatg ttrswctcrg cctc 24

<210> 14
<211> 8
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum

<400> 14
Glu Ala Glu Ser Asn Met Asn Asp
1 5

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

<400> 15
gggtgtcacc acttgcttgc gttt 24

<210> 16
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 16
tcgagtttcc gacgaaagtg gacga 25

<210> 17
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

<400> 17
ctatcatgtg cacgtactcg gtgtgc 26

<210> 18
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 18
ctgggacggt caaagcacgg tactgc 26

<210> 19
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

<400> 19
cagcgacaac atgagagagc tcg 23

<210> 20
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:forward primer

<400> 20
cgatgaggtc atgtgcctgg ataa 24

<210> 21
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 21
aaacggaggc acgtggtgat g 21

<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 22
cgcgtctatc tcatccattc ctcg 24

<210> 23
<211> 444
<212> PRT
<213> Phytophthora cinnamomi

<400> 23
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Val Asp Pro
20 25 30

Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile Asn
35 40 45

Val Tyr Tyr Asn Glu Ala Thr Gly Gly Arg Tyr Val Pro Arg Ala Ile
50 55 60

Leu Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ala Gly Pro
65 70 75 80

Tyr Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Thr Gly
85 90 95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
100 105 110

Ile Asp Ser Val Leu Asp Val Val Arg Lys Glu Ala Glu Ser Cys Asp
115 120 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
130 135 140

Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro
145 150 155 160

Asp Arg Ile Met Cys Thr Tyr Ser Val Cys Pro Ser Pro Lys Val Ser
165 170 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
180 185 190

Val Glu Asn Ala Asp Glu Val Met Cys Leu Asp Asn Glu Ala Leu Tyr
195 200 205

Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Asn Pro Thr Tyr Gly Asp
210 215 220

Leu Asn His Leu Val Cys Ala Ala Met Ser Gly Ile Thr Thr Cys Leu
225 230 235 240

Arg Phe Pro Gly Gln Leu Asn Ser Val Leu Lys Leu Phe Ala Val Asn
245 250 255

Leu Ile Pro Phe Pro Arg Leu His Phe Phe Met Ile Gly Phe Ala Pro
260 265 270

Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro Glu
275 280 285

Leu Thr Gln Gln Gln Phe Asp Ala Lys Asn Met Met Cys Ala Ala Asp
290 295 300

Pro Arg His Gly Arg Tyr Leu Thr Ala Ala Cys Met Phe Arg Gly Arg
305 310 315 320

Met Ser Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys
325 330 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile Lys Ala Ser
340 345 350

Val Cys Asp Ile Pro Pro Gln Gly Leu Lys Met Ser Thr Thr Phe Ile
355 360 365

Gly Asn Ser Thr Ala Ile Gln Glu Met Phe Lys Arg Val Ser Glu Gln
370 375 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385 390 395 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
405 410 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Gly Thr Ala Glu Glu
420 425 430

Glu Gly Glu Phe Asp Glu Asp Glu Glu Trp Met Arg
435 440

<210> 24
<211> 445
<212> PRT
<213> Homo sapiens

<400> 24

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Gly Lys Ala Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp Pro
             20                  25                  30

Thr Gly Thr Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile Asn
         35                  40                  45

Val Tyr Tyr Asn Glu Ala Thr Gly Gly Lys Tyr Val Pro Arg Ala Val
     50                  55                  60

Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ser Gly Pro
 65                  70                  75                  80

Phe Gly Gln Ile Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser Gly
                 85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Val Asp Ser Val Leu Asp Val Val Arg Lys Glu Ala Glu Ser Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Leu Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro
145                 150                 155                 160

Asp Arg Ile Met Asn Thr Phe Ser Val Val Pro Ser Pro Lys Val Ser
                165                 170                 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
            180                 185                 190

Val Glu Asn Thr Asp Glu Thr Tyr Cys Ile Asp Asn Glu Ala Leu Tyr
        195                 200                 205

Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp
    210                 215                 220

Leu Asn His Leu Val Ser Ala Thr Met Ser Gly Val Thr Thr Cys Leu
225                 230                 235                 240

Arg Phe Pro Gly Gln Leu Asn Ala Asp Leu Arg Lys Leu Ala Val Asn
                245                 250                 255
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Met Val Pro Phe Pro Arg Leu His Phe Phe Met Pro Gly Phe Ala Pro
            260                 265                 270

Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro Glu
        275                 280                 285

Leu Thr Gln Gln Met Phe Asp Ala Lys Asn Met Met Ala Ala Cys Asp
    290                 295                 300

Pro Arg His Gly Arg Tyr Leu Thr Val Ala Ala Val Phe Arg Gly Arg
305                 310                 315                 320

Met Ser Met Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys
                325                 330                 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Val Lys Thr Ala
            340                 345                 350

Val Cys Asp Ile Pro Pro Arg Gly Leu Lys Met Ser Ala Thr Phe Ile
        355                 360                 365

Gly Asn Ser Thr Ala Ile Gln Glu Leu Phe Lys Arg Ile Ser Glu Gln
    370                 375                 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385                 390                 395                 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
                405                 410                 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala Glu Glu
            420                 425                 430

Glu Gly Glu Phe Glu Glu Glu Ala Glu Glu Glu Val Ala
        435                 440                 445
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<210> 25
<211> 447
<212> PRT
<213> Neurospora crassa

<400> 25

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Gly Ala Ala Phe Trp Gln Thr Ile Ser Gly Glu His Gly Leu Asp Ala
             20                  25                  30

Ser Gly Val Tyr Asn Gly Thr Ser Glu Leu Gln Leu Glu Arg Met Asn
         35                  40                  45

Val Tyr Phe Asn Glu Ala Ser Gly Asn Lys Tyr Val Pro Arg Ala Val
     50                  55                  60
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Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ala Val Arg Ala Gly Pro
 65                  70                  75                  80

Phe Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser Gly
                 85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Val Asp Gln Val Leu Asp Val Val Arg Arg Glu Ala Glu Gly Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ala Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Phe Pro
145                 150                 155                 160

Asp Arg Met Met Ala Thr Phe Ser Val Val Pro Ser Pro Lys Val Ser
                165                 170                 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
            180                 185                 190

Val Glu Asn Ser Asp Glu Thr Phe Cys Ile Asp Asn Glu Ala Leu Tyr
        195                 200                 205

Asp Ile Cys Met Arg Thr Leu Lys Leu Ser Asn Pro Ser Tyr Gly Asp
    210                 215                 220

Leu Asn His Leu Val Ser Ala Val Met Ser Gly Val Thr Val Ser Leu
225                 230                 235                 240

Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu Ala Val Asn
                245                 250                 255

Met Val Pro Phe Pro Arg Leu His Phe Phe Met Val Gly Phe Ala Pro
            260                 265                 270

Leu Thr Ser Arg Gly Ala His His Phe Arg Ala Val Ser Val Pro Glu
        275                 280                 285

Leu Thr Gln Gln Met Phe Asp Pro Lys Asn Met Met Ala Ala Ser Asp
    290                 295                 300

Phe Arg Asn Gly Arg Tyr Leu Thr Cys Ser Ala Ile Phe Arg Gly Lys
305                 310                 315                 320

Val Ser Met Lys Glu Val Glu Asp Gln Met Arg Asn Val Gln Asn Lys
                325                 330                 335

Asn Ser Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Val Gln Thr Ala
            340                 345                 350
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Leu Cys Ser Ile Pro Pro Arg Gly Leu Lys Met Ser Ser Thr Phe Val
        355                 360                 365

Gly Asn Ser Thr Ala Ile Gln Glu Leu Phe Lys Arg Ile Gly Glu Gln
    370                 375                 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385                 390                 395                 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
                405                 410                 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Gly Val Asp Glu
            420                 425                 430

Glu Glu Glu Glu Tyr Glu Glu Glu Ala Pro Leu Glu Gly Glu Glu
        435                 440                 445


<210> 26
<211> 447
<212> PRT
<213> Aspergillus nidulans

<400> 26
Met Arg Glu Ile Val His Leu Gln Thr Gly Gln Cys Gly Asn Gln Ile
  1               5                  10                  15

Gly Ala Ala Phe Trp Gln Thr Ile Ser Gly Glu His Gly Leu Asp Gly
            20                  25                  30

Ser Gly Val Tyr Asn Gly Thr Ser Asp Leu Gln Leu Glu Arg Met Asn
        35                  40                  45

Val Tyr Phe Asn Glu Ala Ser Gly Asn Lys Tyr Val Pro Arg Ala Val
    50                  55                  60

Leu Val Asp Leu Glu Pro Gly Thr Met Asp Cys Val Arg Ala Gly Pro
65                  70                  75                  80

Phe Gly Glu Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Ser Gly
                85                  90                  95

Ala Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu
            100                 105                 110

Val Asp Asn Val Val Asp Val Val Arg Arg Glu Ala Glu Gly Cys Asp
        115                 120                 125

Cys Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly
    130                 135                 140

Ala Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Phe Pro
145                 150                 155                 160
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Asp Arg Met Met Ala Thr Phe Ser Val Val Pro Ser Pro Lys Val Ser
165 170 175

Asp Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu
180 185 190

Val Glu His Ser Asp Glu Thr Phe Cys Ile Asp Asn Glu Ala Leu Tyr
195 200 205

Asp Ile Cys Met Arg Thr Leu Lys Leu Ser Asn Pro Ser Tyr Gly Asp
210 215 220

Leu Asn His Leu Val Ser Ala Val Met Ser Gly Val Thr Thr Cys Leu
225 230 235 240

Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Trp Ala Val Asn
245 250 255

Met Val Pro Phe Pro Arg Leu His Phe Phe Met Val Gly Phe Ala Pro
260 265 270

Leu Thr Ser Arg Gly Ala Tyr Ser Phe Arg Ala Val Ser Val Pro Glu
275 280 285

Leu Thr Gln Gln Met Phe Asp Pro Lys Asn Met Met Ala Ala Ser Asp
290 295 300

Phe Arg Asn Gly Arg Tyr Leu Thr Cys Ser Ala Ile Phe Arg Gly Lys
305 310 315 320

Val Ser Met Lys Glu Val Glu Asp Gln Met Arg Asn Ile Gln Ser Lys
325 330 335

Asn Gln Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile Gln Ser Ala
340 345 350

Leu Cys Ser Ile Pro Pro Arg Gly Leu Lys Met Ser Ser Thr Phe Ile
355 360 365

Gly Asn Ser Thr Ser Ile Gln Glu Leu Phe Lys Arg Val Gly Asp Gln
370 375 380

Phe Thr Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly
385 390 395 400

Glu Gly Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn
405 410 415

Asp Leu Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Ser Ile Ser Glu
420 425 430

Gly Glu Glu Glu Tyr Ala Glu Glu Glu Ile Met Glu Gly Glu Glu
435 440 445

<210> 27
<211> 444
<212> PRT
<213> Achlya klebsiana

<400> 27
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Val Asp Pro
20 25 30

Thr Gly Ser Tyr His Gly Asp Ser Asp Leu Gln Leu Glu Arg Ile Asn
35 40 45

Val Tyr Tyr Asn Glu Ala Thr Gly Thr Tyr Val Pro Arg Ala Ile Leu
50 55 60

Met Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ala Gly Pro Tyr
65 70 75 80

Gly Gln Leu Phe Arg Pro Asp Asn Phe Val Phe Gly Gln Thr Gly Ala
85 90 95

Gly Asn Asn Trp Ala Lys Gly His Tyr Thr Glu Gly Ala Glu Leu Ile
100 105 110

Asp Ser Val Leu Asp Val Val Arg Lys Glu Ala Glu Ser Cys Asp Cys
115 120 125

Leu Gln Gly Phe Gln Ile Thr His Ser Leu Gly Gly Gly Thr Gly Ser
130 135 140

Gly Met Gly Thr Leu Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro Asp
145 150 155 160

Arg Ile Met Cys Thr Tyr Ser Val Cys Pro Ser Pro Lys Val Ser Asp
165 170 175

Thr Val Val Glu Pro Tyr Asn Ala Thr Leu Ser Val His Gln Leu Val
180 185 190

Glu Asn Ala Asp Glu Val Met Cys Leu Asp Asn Glu Ala Leu Tyr Asp
195 200 205

Ile Cys Phe Arg Thr Leu Lys Leu Thr Asn Pro Thr Tyr Gly Asp Leu
210 215 220

Asn His Leu Val Cys Ala Ala Met Ser Gly Ile Thr Thr Leu Leu Arg
225 230 235 240

Phe Pro Gly Gln Leu Asn Ser Val Leu Lys Leu Ala Val Asn Leu Ile
245 250 255

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Pro Phe Pro Arg Leu His Phe Phe Met Ile Gly Phe Ala Pro Leu Thr
            260                 265                 270

Ser Arg Gly Ser Gln Gln Tyr Arg Ala Leu Thr Val Pro Glu Leu Thr
        275                 280                 285

Gln Gln Gln Phe Asp Ala Lys Asn Met Met Cys Ala Ala Asp Pro Arg
    290                 295                 300

His Gly Arg Tyr Leu Thr Ala Ala Cys Met Phe Arg Gly Arg Met Ser
305                 310                 315                 320

Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys Asn Ser
                325                 330                 335

Ser Tyr Phe Val Glu Trp Ile Pro Asn Asn Ile Lys Ala Ser Val Cys
            340                 345                 350

Asp Ile Pro Pro Lys Gly Leu Lys Met Ser Thr Thr Phe Ile Gly Asn
        355                 360                 365

Ser Thr Ala Ile Gln Glu Met Phe Lys Arg Val Ser Glu Gln Phe Thr
    370                 375                 380

Ala Met Phe Arg Arg Lys Ala Phe Leu His Trp Tyr Thr Gly Glu Gly
385                 390                 395                 400

Met Asp Glu Met Glu Phe Thr Glu Ala Glu Ser Asn Met Asn Asp Leu
                405                 410                 415

Val Ser Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala Glu Glu Glu Gly
            420                 425                 430

Glu Phe Asp Glu Asp Glu Glu Met Asp Glu Met Met
        435                 440
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<210> 28
<211> 15
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans, and A. klebsiana

<400> 28

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Ala Ile Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg
  1               5                  10                  15
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<210> 29
<211> 15
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans, and A. klebsiana

<400> 29
Ala Val Leu Val Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg
1 5 10 15

<210> 30
<211> 7
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans, and A. klebsiana

<400> 30
Gly Gly Gly Thr Gly Ser Gly
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<210> 31
<211> 4
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans, and A. klebsiana

<400> 31
Asp Asn Glu Ala
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<210> 32
<211> 4
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans and A. klebsiana

<400> 32
Met Arg Glu Ile
1

<210> 33
<211> 4
<212> PRT
<213> Pestalotiopsis microspora

<220>
<223> motif conserved in P. ultimum, H. sapiens, N. crassa, A. nidulans, and A. klebsiana

<400> 33
Met Arg Glu Leu
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<210> 34
<211> 31
<212> PRT
<213> Sus scrofa

<400> 34
Met Arg Glu Ile Val His Ile Gln Ala Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 35
<211> 20
<212> PRT
<213> Sus scrofa

<400> 35
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 36
<211> 31
<212> PRT
<213> Homo sapiens

<400> 36
Met Arg Glu Ile Val His Leu Gln Ala Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 37
<211> 20
<212> PRT
<213> Homo sapiens

<400> 37
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 38
<211> 31
<212> PRT
<213> Drosophila melanogaster

<400> 38
Met Arg Glu Ile Val His Ile Gln Ala Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Ile Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 39
<211> 20
<212> PRT
<213> Drosophila melanogaster

<400> 39
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Leu
20

<210> 40
<211> 31
<212> PRT
<213> Xenopus laevis

<400> 40
Met Arg Glu Ile Val His Leu Gln Ala Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 41
<211> 20
<212> PRT
<213> Xenopus laevis

<400> 41
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 42
<211> 31
<212> PRT
<213> Tetrahymena thermophila

<400> 42
Met Arg Glu Ile Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 43
<211> 20
<212> PRT
<213> Tetrahymena thermophila

<400> 43
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 44
<211> 31
<212> PRT
<213> Physarum polycephalum

<400> 44

Met Arg Glu Ile Val His Ile Gln Ala Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Ile Asp
20 25 30

<210> 45
<211> 20
<212> PRT
<213> Physarum polycephalum

<400> 45
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 46
<211> 31
<212> PRT
<213> Pythium ultimum

<400> 46
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Val Asp
20 25 30

<210> 47
<211> 20
<212> PRT
<213> Pythium ultimum

<400> 47
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15
Leu Val Cys Ala
20

<210> 48
<211> 31
<212> PRT
<213> Phytophthora cinnamomi

<400> 48
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Val Ser Asp Glu His Gly Val Asp
20 25 30

<210> 49
<211> 20
<212> PRT
<213> Phytophthora cinnamomi

<400> 49
Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Cys Ala
20

<210> 50
<211> 31
<212> PRT
<213> Achlya klebsiana

<400> 50
Met Arg Glu Leu Val His Ile Gln Gly Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Lys Phe Trp Glu Val Ile Ser Asp Glu His Gly Val Asp
20 25 30

<210> 51
<211> 20
<212> PRT
<213> Achlya klebsiana

<400> 51
Phe Arg Thr Leu Lys Leu Thr Asn Pro Thr Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Cys Ala
20

<210> 52
<211> 31
<212> PRT
<213> Pestalotiopsis microspora

<400> 52
Met Arg Glu Ile Val His Leu Gln Thr Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Ala Phe Trp Gln Thr Ile Ser Gly Glu His Gly Leu Asp
20 25 30

<210> 53
<211> 20
<212> PRT
<213> Pestalotiopsis microspora

<400> 53
Met Arg Thr Leu Lys Leu Ser Asn Pro Ser Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 54
<211> 31
<212> PRT
<213> Aspergillus nidulans

<400> 54
Met Arg Glu Ile Val His Leu Gln Thr Gly Gln Cys Gly Asn Gln Ile
1 5 10 15

Gly Ala Ala Phe Trp Gln Thr Ile Ser Gly Glu His Gly Leu Asp
20 25 30

<210> 55
<211> 20
<212> PRT
<213> Aspergillus nidulans

<400> 55
Met Arg Thr Leu Lys Leu Ser Asn Pro Ser Tyr Gly Asp Leu Asn His
1 5 10 15

Leu Val Ser Ala
20

<210> 56
<211> 31
<212> PRT
<213> Saccharomyces cerevisiae

<400> 56
Met Arg Glu Ile Ile His Ile Ser Ala Gly Gln Tyr Gly Asn Gln Ile
1 5 10 15

Gly Ala Ala Phe Trp Glu Thr Ile Cys Gly Glu His Gly Leu Asp
20 25 30

<210> 57
<211> 20
<212> PRT
<213> Saccharomyces cerevisiae

<400> 57
Gln Arg Thr Leu Lys Leu Asn Gln Pro Ser Tyr Gly Asp Leu Asn Asn
1 5 10 15

Leu Val Ser Ser
20

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 00/07995

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/37 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, BIOTECHNOLOGY ABS, SCISEARCH, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEERAKOON N D ET AL: "Isolation and characterization of the single beta-tubulin gene in Phytophthora cinnamomi." MYCOLOGIA, vol. 90, no. 1, January 1998 (1998-01), pages 85-95, XP000929307 ISSN: 0027-5514 cited in the application	55-67, 70-72, 78,79
Y	the whole document	1-9, 11-20, 24-26, 28-36, 38-49, 51-53, 74-77, 80-84

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2000	08/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

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INTERNATIONAL SEARCH REPORT

In. ational Application No
PCT/US 00/07995

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LONG DAVID M ET AL: "In vivo addition of telomeric repeats to foreign DNA generates extrachromosomal DNAs in the taxol-producing fungus Pestalotiopsis microspora." FUNGAL GENETICS AND BIOLOGY, vol. 24, no. 3, August 1998 (1998-08), pages 335-344, XP000929414 ISSN: 1087-1845 the whole document	1-9, 11-20, 24-26, 74,75, 80-82,97
Y	YOUNG D H ET AL: "Antifungal properties of taxol and various analogues." EXPERIENTIA (BASEL), vol. 48, no. 9, 1992, pages 882-885, XP000929422 ISSN: 0014-4754 cited in the application the whole document	28-36, 38-49, 51-53, 76,77, 83,84,97
Y	RAO SRINIVASA ET AL: "Characterization of the Taxol Binding Site on the Microtubule: 2-(m-azidobenzoyl)taxol photolabels a peptide (amino acids 217-231) of beta-tubulin." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 35, 1995, pages 20235-20238, XP002143078 ISSN: 0021-9258 cited in the application the whole document	1-9, 11-20, 24-26
A	NOGALES EVA ET AL: "Structure of the alphabeta tubulin dimer by electron crystallography." NATURE (LONDON), vol. 391, no. 6663, 8 January 1998 (1998-01-08), pages 199-203, XP002143079 ISSN: 0028-0836 the whole document	1-7, 11-20, 24-26
A	" Acremonium chrysogenum wild-type beta-tubulin." GENESEQ DATABASE ; ACESSION NUMBER R40226 ; JP5192157, XP002143080 the whole document	1-7, 11-20, 24-26

-/--

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/07995

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MU J -H ET AL: "Analysis of beta-tubulin cDNAs from taxol-resistant Pestalotiopsis microspora and taxol-sensitive Pythium ultimum and comparison of the taxol-binding properties of their products." MOLECULAR AND GENERAL GENETICS, vol. 262, no. 4-5, December 1999 (1999-12), pages 857-868, XP002143081 ISSN: 0026-8925 the whole document	1-54

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